

A tale of two budget systems

The United States may have something to learn from Britain's different handling of the economy, but the effects of Britain's entry into the European Monetary System will not be known for some time.

THE founding fathers of the United States, with the Boston Tea Party's slogan ringing in their ears, cannot have imagined a time when there would be no taxation even with representation. Yet that is what last weekend's farce in Washington shows now to be the case. When the House of Representatives last Thursday threw out the budget bitterly negotiated between the White House and the Congress over four painful months, its members were in effect complaining that it is more than can be expected of flesh and blood to require the people's representatives to face an election (a month from now) when they have recently given assent to measures that may be unpopular with many voters. No crisis, they were saying, can be worse than not being re-elected.

There may have been some regret in the United States, at the White House in particular, at last weekend's demonstration that in Britain, which provoked the Boston business, matters are still ordered differently. With no specific word of warning, the Chancellor of the Exchequer, Mr John Major, declared that Britain would promptly become an effective member of the European Monetary System — probably the most significant change in Britain's monetary constitution since the abandonment of the Gold Standard in 1931. Again there are electoral calculations, but in a system in which governments themselves decide when to stand for re-election, only the unfolding of events will show what these may have been.

The US budget dispute is the more serious because the US economy is more influential than the British, because the direct effects of a failure to resolve it will be directly harmful, not least to the welfare of the scientific enterprise in the United States — and because there is also a danger that a solution, if unintelligently put into effect, may itself have consequences that are calamitous.

The outline of the crisis is well known — the federal government outspends its revenue by between \$150,000 million and twice as much, depending on how (and by whom) the arithmetic is done. For the past half decade, the Congress has bound itself either to effect regular reductions of the deficit or to have government spending cut indiscriminately across the board. After promising during the 1989 election that taxes would never be increased, and having eaten humble pie, President George Bush is now rightly demanding that the Congress should give him a budget, not sequestration by default.

On that the president has no choice. The deficits of the

Reagan years were a device for making people in the United States feel good, able to enjoy public services they had not paid for. The sleight of hand depended on the willingness of people elsewhere either to lend directly to the US government or to buy tangible assets in the United States, releasing dollars for the US Treasury to borrow. But that source of funds has now dried up.

The whole world is short of money, and US dollars are cheaper than they have been for years. Yet the United States is heading for recession, when a federal deficit would make keynesian sense, as would the further reduction of interest rates to which the Federal Reserve is edging. Has Bush's devotion to a budget settlement, which would have been proper in any of the ten years past and perhaps even when this year's budget cycle began in February, been overtaken by events? The United States would find a serious recession uncomfortable, but many among the rest of us would find it calamitous as well.

At least in some procedural respects, the British government's problem has something in common with that in Washington. As in the United States, economic activity is rapidly decelerating, but the government is having to squeeze excess credit out of the system by means of high interest rates, upwards of 15 per cent until last Friday. They could not have been allowed to rise much higher without exciting the wrath of voters, and might have been economically dangerous as well. So why not join the Exchange Rate Mechanism, the system by which the European Monetary System ensures that the currencies of the European Communities' members remain more or less fixed in relation to each other?

The decision to do just this, pulled like a rabbit from a hat on the eve of the government's annual meeting with its party supporters, has already been used to bring down interest rates by 1 per cent. That benefit, probably the first of several of the kind, is immediate. The price that will have to be paid — the painful business of keeping inflation within reasonable bounds — will on the other hand be delayed by several months. There are piquant side-issues, not least the prime minister's much advertised coolness to this scheme and the possibility that, in the changed circumstances, Major may be better able to make his sensible version of the case for a single European currency. Nevertheless, Bush is fighting to resist externally imposed sequestration just when Major is tying Britain to an external source of discipline. □



NIH launches surprise Gallo investigation

- Existence of independent viruses accepted
- Missing data and misleading statements

London & Washington

IN A surprise announcement, the National Institutes of Health (NIH) last week said that it had decided to convert its inquiry into the conduct of National Cancer Institute researcher Robert Gallo into a "formal investigation", even though it had resolved or "shown to be without substance" several of the "publicized issues and allegations" concerning Gallo's role in the discovery of the AIDS virus.

According to a confidential letter sent by NIH acting director William Raub to Gallo, findings to date indicate that there is "substantial reason to believe that scientific misconduct may have occurred in some instances" during the fiercely competitive hunt for the AIDS virus in 1983 and 1984.

But the specifics of the coming investigation, given in the confidential letter to Gallo, appear to indicate that any "misconduct" involves issues of misleading wording and missing data, rather than the broad issue that Gallo "stole" the virus from competing researcher Luc Montagnier of the Pasteur Institute in Paris.

Vanishing motive

According to Gallo, the issue has now "been conclusively taken care of" because the inquiry accepts that his laboratory had isolates of AIDS virus from sources other than the Pasteur Institute at the time the virus was being identified as the cause of AIDS. Raub's statement says that "the inquiry team has concluded that Dr Gallo had a substantial number of HIV detections and isolations from several different sources at the critical time . . .". With viruses from other sources growing in his laboratory, Gallo claims that he would have no reason to steal the French virus, known as LAV.

Gallo is, however, willing to accept that the extreme similarity of LAV and the virus he isolated — named HTLV-IIIB — could indicate that LAV entered his own culture through accidental contamination. But viral contamination is a common problem and not, says Gallo, "a question of ethics".

The NIH investigation will attempt to resolve the issue of where Gallo's virus came from. According to Raub's prepared statement, the investigation is to "include testing of a number of biological samples in an effort to determine the origins of HTLV-IIIB, the virus that Dr Gallo and his colleagues used to develop the [AIDS] blood test." Gallo says that the testing

process will attempt to determine whether any of the 10 viruses known to have been independently isolated in his laboratory at the time could have provided the source of HTLV-IIIB. The investigation will also include Gallo's chief virologist, Mikulas Popovic, who had been responsible for growing viruses during the critical 1983–84 period.

Gallo's lawyer, Joseph Onek, says that the fact that NIH officials are addressing the issue at all proves that they consider it an open question. "When you're saying that you're looking for the source of HTLV-IIIB that means you are not necessarily accepting contamination", but are considering the possibility of independent isolations, he says.

According to Gallo, it may be difficult for NIH to come up with a final judgement on the origin of his virus. Although only 10 separate viruses were known to have been combined in the viral "pool" maintained by Popovic from which HTLV-IIIB was derived, some 78 other viral isolates were known to be in the laboratory at the time — and contamination from them is as likely as from LAV, says Gallo. Also, two of the original 10 isolates that went into the pool are no longer in existence, so their similarity to HTLV-IIIB/LAV cannot be checked.

The issue of missing data to be raised in the formal investigation focuses on a paper by Popovic and Gallo published in May 1984. The paper was the first of a series of four key reports published by Gallo's laboratory in a single issue of *Science*; taken together, they served to establish HTLV-IIIB as the cause of AIDS.

John Crewdson, the investigative reporter whose 16-page article in the *Chicago Tribune* newspaper stimulated the NIH inquiry, alleges that there are important discrepancies between the information published in the paper and Popovic's laboratory notes. In particular, he maintains that a table describing the properties of 5 viral isolates, growing in Gallo's laboratory before publication is misleading.

The questions about the paper bear strongly on the precise status of various viral isolates that were being kept in Gallo's laboratory at the time, and in particular on whether Popovic grew LAV in continuous rather than transient cell cultures. As it happens, it was Crewdson's attempts to resolve questions of this type that led him to argue that HTLV-IIIB was

derived from LAV rather than an independent isolate.

Once again though, a final answer may be impossible to obtain, largely because many records, especially those kept by Popovic, no longer exist. "The real problem is that Popovic never kept a notebook, period," says Gallo. (Popovic was in his native Czechoslovakia last week and could not be reached.) Given the increasingly rigid standards for laboratory records being set as a result of other notorious misconduct cases, "the missing data could be a problem" in the investigation, says Gallo. "We are in new times."

In his statement, Raub announced that the NIH Office of Scientific Integrity which had handled the inquiry, would also undertake the formal investigation with a yet-unnamed panel of "expert scientific advisors" and the continued oversight and guidance of an 11-person panel nominated by the National Academy of Science (NAS).

Missing data

This summer, the NAS panel wrote to Raub, saying that they had found that "some data appears to be missing from the data books . . . [and] there is a possibility of selection and/or misrepresentation of data." They requested that the inquiry be terminated and a full investigation begun at that time. But Raub declined, explaining that "redesignation now would be premature" given that the "inquiry is in an especially significant phase". But what, exactly, was learned between that time and last week remains unclear.

Gallo says the announcement caught him by surprise both by its timing and its conclusion. "I expected this to be finished long ago. I think the major questions of anybody taking anything is over." One of the key allegations raised by Crewdson was that Gallo attempted to take full credit for the discovery of the AIDS virus even after he was familiar with Montagnier's work. Gallo acknowledges that investigators may still conclude that his papers show "exaggeration in the interest of petty gain", but that is an interpretation he believes is due to sloppy paper-writing in a tense period. "It was an emergency time. We weren't worried about dotting the 'i's and crossing the 't's" he says.

Representative John Dingell, chairman of the Energy and Commerce investigations subcommittee that asked NIH to undertake the inquiry, said in a statement that "we intend to follow [NIH's] investigation closely, and will also give them an opportunity to explain to the subcommittee why they have chosen not to pursue all of the allegations" that were most thoroughly aired in Crewdson's article last year.

**Christopher Anderson,
David Concar & Alun Anderson**

Ulysses treads a lonely path

London

AFTER a successful launch and deployment from the space shuttle Discovery on 6 October, the Ulysses solar probe is now speeding towards Jupiter on the first leg of a five-year voyage to fly over both poles of the Sun. The mission is still a collaboration between the European Space Agency (ESA) and the US National Aeronautics and Space Administration (NASA), even though a bitter taste remains from NASA's sudden cancellation of a companion probe to Ulysses in 1981.

Ulysses will be the first spaceprobe to fly over the Sun's poles, taking measurements of the solar wind (see *Nature* 347, 439; 4 October 1990), its associated magnetic field patterns and the Sun's output of X-rays, radio and plasma waves. Until now, all solar observations have been made from the same ecliptic plane in which the Earth orbits the Sun. Spacecraft launched from Earth are flung out in this plane automatically, because of the Earth's own movement, and there is no launch vehicle sufficiently powerful to put Ulysses directly into a solar polar orbit. To overcome the problem, Ulysses will make a 'gravity assist manoeuvre' around Jupiter in February 1992 (similar to those made by Voyager 2 during its tour of the outer planets), stealing energy from the planet to send it in a southern trajectory back towards the Sun, culminating in a south polar flypast in the summer of 1994. Ulysses will then swing round the Sun, flying over the north pole one year later.

Richard Marsden, ESA deputy project scientist, is enthusiastic about the complement of instruments flying on Ulysses, picking out the measurement of heavy element ions in the solar wind and the detection of cosmic rays from outside the Solar System as particularly promising experiments. But Marsden says the loss of the NASA probe will cause "certain problems in interpreting the data".

In the original plan, two probes would have flown past opposite poles simultaneously, allowing both to be studied at the same time with a core payload of instruments shared by the two craft. Ulysses will observe both poles, but it will now be difficult to tell if observed differences between the north and south poles are due to inherent differences between the two or to variation in the Sun's activity in time. Observations using an X-ray/extreme ultraviolet telescope and of the Sun's corona were to be made from the companion craft and are now lost completely.

Joan Johnson-Freese, from the Center for Space Policy and Law at the University of Central Florida, says that NASA's contribution to what was then known as the International Solar Polar Mission (ISPM) was the victim of a "unique set of circum-

stances". In 1981, President Ronald Reagan's new administration was making deep cuts throughout the US budget, while NASA was stripping resources from other space programmes to meet the space shuttle's ballooning costs. NASA was told that one project from the ISPM, the Hubble Space Telescope or the Galileo Jupiter probe, would have to be cut.

But it was not NASA's cancellation of the second ISPM probe that angered ESA officials so much as the lack of consultation before the announcement. Johnson-Freese says ESA and other collaborators were left asking themselves the question: "what good is a memorandum of understanding?", if US budgetary squeezes can jeopardize collaborative projects. She says this has coloured recent negotiations on collaborations such as the planned Freedom space station and the Earth Observing System remote-sensing network.

Ian Pryke, head of the ESA office in Washington, maintains that ESA's relations with NASA are now back on course after the "low point" of the early 1980s. But ESA has little choice but to collaborate with NASA on expensive space science projects, given the limits of its own science budget. Ironically, Willis Meeks, NASA project manager for Ulysses, says the savings achieved through the cancellation were "probably nil". NASA is still paying for two-thirds of the \$750-million mission, supplying the expensive shuttle launch, the booster rocket to power Ulysses towards Jupiter and use of its Deep Space Network. Any savings have been dwarfed by cost overruns because of re-

peated launch delays.

The original launch date was February 1983, but NASA was forced to delay by two years in 1981, to allow that year's budget to balance (despite the fact that this increased overall cost), and the 1986 Challenger disaster caused further setbacks.

Johnson-Freese says ISPM was the victim of the US budgetary system, where government spending is determined year by year. This means that agencies such as NASA and the National Science Foundation (NSF) cannot guarantee a continued level of funding for their side of agreed international 'big science' projects.

D. Allan Bromley, science adviser to the US president, has stressed the foreign perception of the United States as an "unreliable partner" in many of his speeches, and called for "something more like treaty arrangements" to govern international projects.

One solution would be longer-term earmarking of funds for important collaborations. But Dick Malow, a member of staff of the House of Representatives appropriations subcommittee that handles the NASA and NSF budgets, says the US presidential system of government, where members of the House of Representatives are re-elected every two years, precludes this change. Other congressional staff turn criticism of the US budgetary system back on NASA, claiming that NASA has repeatedly given low cost estimates for 'new start' projects and then expected Congress to pay for cost overruns. The same staff say that NASA is still guilty of a lack of realism, asking for an annual budget growth of 15 per cent in real terms in the face of the huge current US budget deficit.

Peter Aldhous

GENETIC ENGINEERING

Glasnost for UK release information

London

BOWING to pressure from environmentalist groups and members of the House of Lords, the UK government is to amend the Environmental Protection Bill so as to ensure public access to information on proposed releases of genetically engineered organisms. The government had originally argued that clauses on public access were not needed because the Environment Secretary already has the power to make this information public.

The new clauses propose public registers containing applications for consents to release, supporting information supplied with these applications, and any advice given to the Environment Secretary by the new independent Advisory Committee on Releases to the Environment (ACRE). Some information may be withheld if the Environment Secretary decides that this would undermine national security, provoke sabotage by extremist groups which would present an environ-

mental hazard, or breach commercial confidentiality. But in commercially sensitive cases, the name and address of the applicant, a description of the organism and the purpose of the release, and the results of any assessment of the environmental risks posed must still be included.

Julian Kinderlerer, from the University of Sheffield, a member of ACRE who has been advising the opposition Labour party on the release of engineered organisms, says he is "thrilled" by the amendments, and surprised at how far the government has gone. The publication of ACRE's advice is particularly significant, he says, because it puts the onus on the Environment Secretary to justify any decisions that go against ACRE's advice. A further amendment makes ACRE a statutory committee, so that a decision of parliament would be needed to abolish it or alter its status. The new amendments will be discussed in the House of Lords next week, at the bill's report stage. Peter Aldhous

Pass the gas masks, please

Munich

NEW revelations that German companies have helped virtually every area of Iraqi military development, including its chemical weapons programme, are continuing to rock the government of Chancellor Helmut Kohl. Rather than shipping weapons themselves, it now appears that German companies concentrated on exporting seemingly harmless goods or machinery that could easily be used to make weapons. Among the allegations are that German companies built nerve and mustard gas factories, helped Iraq to enhance its nuclear and biological weapons programmes, built factories for advanced conventional weapons and helped in the attempt to construct the infamous 150-metre-long giant cannon.

In the latest development, a manager and an engineer from two West German companies confessed last week that they had known that they were helping Iraq to build factories that can be used to produce poison gas. Previously they had contended that the factories were to be used for pesticide production (see *Nature* **332**, 573; 1988).

The West German government acknowledged the huge range of shipments in a secret briefing given by Economics Minister Helmut Haussmann to the Economics Committee of the Bundestag in August. According to one committee member, Haussmann admitted that the range of exports was broader than previously suspected and that West German laws would not necessarily be able to prevent future exports, especially of so-called 'dual-use' technology.

Oskar Lafontaine (Social Democrat), who is challenging Kohl (Christian Democrat) for the post of chancellor in the December elections, declared in a speech before the first meeting of an all-German parliament on 3 October that "it is our responsibility to change the German weapons export policy. We in a new, larger Germany cannot go on [selling] first the gas to Iraq, then the gas masks to Saudi Arabia . . ." West Germany came under severe criticism from the United States last year for its export to Libya of a poison gas plant. The export embarrassed Bonn and caused it to strengthen the laws regulating export of weapons technology, as well as to pursue violators more aggressively. German law is now stricter than that of other European countries and prosecutors are investigating more than 170 companies in 37 separate proceedings, according to the news magazine *Der Spiegel*.

But it took pressure from the opposition Social Democrats to give the new laws any bite. As passed by the Christian-Democrat-controlled Bundestag, the

amended laws would not have increased above one year the minimum criminal penalty for violations. The Bundesrat (upper house), which is now controlled by the Social Democrats, forced minimum penalties of two years and more.

The difference is significant, according to Michael Brzoska, an expert on arms control at the University of Hamburg. Traditionally, he says, defendants given one-year sentences are released on probation by German courts. Public prosecutors normally do not expend the tremendous effort necessary to make a case against exporters unless they expect the defendant, if found guilty, to go to prison.

Brzoska, who has written several books on arms exports, says that Bonn deserves

FOREIGN RESEARCHERS

Asking for trouble

Paris

A SMALL spin-off from the Persian Gulf crisis is causing trouble in the French national research organization, CNRS. First, a government directive was issued asking for the names of all visiting scientists from Iraq. Then Claude Paoletti, director of the life sciences section of CNRS, sent a letter to heads of the 300 CNRS life sciences laboratories asking for the names not just of Iraqis, but also of researchers from Kuwait, Iran, Turkey, Morocco, Algeria, Tunisia, Egypt, Libya, Syria, Jordan, Lebanon and Pakistan. The affair has now caught the eye of the Paris-based League for Human Rights, and Paoletti's action, apparently taken on his own initiative, has brought more opprobrium to an already controversial character (see *Nature* **339**, 326; 1989).

According to Rose Katz, secretary general of SNCS-FEN, the major trade union representing researchers, two Iraqi scientists have already been asked to stay at home. One, a doctoral student in physics, was working on sensitive aerothermal techniques at Meudon, near Paris. The other is a researcher at the national agricultural research institute.

What has particularly irked the union and civil rights bodies is Paoletti's apparently personal decision to extend the scope of the government's request. SNCS-FEN was quick to denounce his request as "discriminatory" and to ask its members to ignore the letter.

Meanwhile, the League for Human Rights has opened a dossier on the affair and has requested the national commission on the freedom of information (CNIL) to investigate the legality of CNRS's action in obtaining and filing the information requested. At the end of

its bad reputation even though other countries, including France and the Soviet Union, exported more weapons to Iraq. Given the size of its economy, he says, "[West] Germany has been proportionally no worse than other countries", but it has made itself conspicuous by ignoring the problem of illegal or dual-use exports. The export authorities did nothing, he says, when clues poured in concerning "hundreds of cases".

Even after the changes in the law, Brzoska says that Germany could be doing a lot more to stop dubious exports. Industry is often given the benefit of the doubt, he says, because "the export laws are written so that business should be disturbed as little as possible." Instead, Brzoska says, the laws should be rewritten so that everything is checked that might be useful for weapons production.

Steven Dickman

September, the director-general of CNRS, François Kourilsky, issued a statement denying accusations of racism, but leaving Paoletti to fend for himself. "Our concern was only to contact Iraqi researchers", says Kourilsky, who added that the request of the life sciences section to update its list of foreign students was "badly conceived in the current climate of the Gulf crisis". But he said there was no discriminatory intent on Paoletti's part.

Paoletti would not comment last week. But at CNRS headquarters in Paris, Jean-François Huchet, responsible for external relations, confirmed that Paoletti's request for details of visitors from other Muslim countries was routine practice, albeit ill-timed. "The files we keep on researchers have all been authorized by the CNIL", said Huchet. "Dr Paoletti anticipated a little, but we have to keep these files up to date." Huchet said that under special circumstances, such as the Gulf crisis, or the massacre of Chinese students in Beijing, the administration may ask for a head-count of students potentially implicated. "I think this is good management", says Huchet. "If we did not know where visiting students are or what their status is, we would be accused of sloppiness".

Some directors of life sciences laboratories have followed union advice and ignored Paoletti's letter. "If he wants to check up on foreign students he should call in the police", said one laboratory director — a reaction that Huchet called "professional misconduct".

Meanwhile, the president of the League of Human Rights is still not satisfied with Kourilsky's explanation and is waiting for the CNIL to give its opinion on the legality of CNRS files.

Peter Coles

A pictorial progress report

Baltimore

SINCE the days of gloom and pessimism that immediately followed the discovery of spherical aberration in the optics of the Hubble Space Telescope (HST), astronomers have become more cheery as they have learned more about what the HST can still do. Last week, during a show put on at the Space Telescope Science Institute in Baltimore to display the latest achievements of the Wide Field/Planetary Camera (WFPC) and Faint Object Camera (FOC), HST director Riccardo Giacconi declared that he was "cheerfully depressed": the telescope has obvious limitations, but in certain applications it is nevertheless far superior to ground-based telescopes, as the images on this page demonstrate.

The key to recent progress has been what some HST scientists call the D-word: deconvolution. HST's optical flaw spreads each point source of light in an image into a blur of known profile. Deconvolution is the mathematical procedure by which the blurring is unfolded from the images to create an 'unblurred' photograph (see *Nature* 346, 686; 1990).

This process can approach perfection for images that consist of a small number of isolated point sources — a loose grouping of stars, for example. But for dense star clusters, where the individual images overlap, and even more for nebulous extended objects whose brightness varies continuously over a large area, deconvolution is less successful; it cannot put back into an image information which the blurring has destroyed. Among last week's presentations, a few outright failures were included. Sandy Faber, of the Lick Observatory in California, con-

cluded after taking some test images that the HST in its present form would not be able to perform one of its most eagerly awaited tasks, that of pinning down the expansion rate of the Universe. An essential step in this measurement was to have

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

In 1938 a nova exploded in the globular cluster M14, and in the photograph (left) a faint star, near the centre of the image, was found which was of unusual colour. It was tentatively identified as the nova in its now quiescent state. But the FOC picture (right) (so much higher in resolution that corresponding images in the two photos can be identified only with difficulty) dispels this idea. In place of the faint nova, as many as a dozen candidate stars appeared, all of normal colour. The unusual colour seen from the ground was due to a conspiracy of ordinary stars getting together to look like one odd one.

been the detection of Cepheid variable stars, whose intrinsic brightness can be deduced from their period of variation, in distant galaxies. Comparison of the measured brightness with the deduced intrinsic brightness then yields an accurate distance with which to calibrate the scale of the Universe. But, as Faber discovered, the brightness of faint Cepheids in distant galaxies cannot be measured with sufficient accuracy on the blurred images, and deconvolution degrades brightness information as it restores spatial resolution.

HST astronomers are now engaged in a science assessment programme (during which the images shown here were obtained) whose purpose is to characterize what the instruments can do, and what they cannot. These findings will be distributed to the astronomers currently on the roster of scheduled observing time so that they can decide whether their planned observations can be done, perhaps with longer exposure times, or if they should be postponed until the HST has been fixed. Early next year, the Telescope Allocation Committee will reconvene to decide on a new schedule of observations.

Despite the spherical aberration, and despite the continuing jitters that still afflict HST when it crosses from day to night and back again, astronomers are clearly happy with what they have done, and will be able to do. Last week's presentation of new images was unabashedly an attempt to show that HST can still do wondrous things. It succeeded.

David Lindley

New research lobby draws fire

Washington

FOUR congressmen last week announced the formation of a 24-member legislative caucus aimed at promoting basic biomedical research and held its first meeting, a seminar on maintaining the United States' leading position in the field. But although the seminar attracted such luminaries as Nobel Laureate Harold Varmus of the University of California and Leon Rosenberg, dean of the Yale University School of Medicine, some leading members of Congress were absent.

Most prominent of the names absent from the caucus lineup is William Natcher (Democrat, Kentucky) chairman of the House of Representatives appropriations subcommittee that controls most of the federal biomedical research funding. Other missing research supporters include full appropriations committee chair Jamie Whitten (Democrat, Mississippi) and minority chair Silvio Conte (Democrat, Massachusetts). "It's surprising to see a biomedical research caucus that doesn't have any of the long-term research champions, especially since these champions already think of themselves as a caucus", says Carol Sheeman of the American Association of Universities.

Christopher Anderson

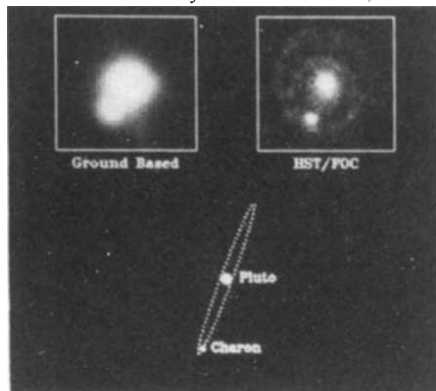
Industry fights back

Washington

US BIOMEDICAL companies and associations are planning to put together a new organization to oppose the public relations efforts of anti-animal research activists, industry officials said last week. Although plans have not been finalized, Anneliz Hannan, director of corporate affairs at US Surgical Corporation and a principal organizer of the project, says a budget of \$17–\$25 million a year will be needed to counter animal activist campaigns adequately. Rather than duplicate the work of existing groups that focus on legislative lobbying, the new organization will serve as an 'umbrella' group, she says.

The new organization will target high schools, either by educating teachers or addressing students directly, Hannan says. An education programme now being sponsored by US Surgical in Connecticut may serve as the model. The American Medical Association, Procter and Gamble, and Squibb Pharmaceuticals are said to be among those interested in setting up the new organization. A possible director of the organization is rumoured to be Frederick Goodwin, currently the administrator of the Alcohol, Drug and Mental Health Administration and an outspoken advocate of animal research.

Christopher Anderson



A simple demonstration of resolving power. The right-hand FOC photograph of Pluto and its large satellite Charon is a "raw" image, with no attempt made to remove the clearly visible halos due to spherical aberration, but is still a clear improvement over the best ground-based image (left).

Industry gets a bargain

London

BRITISH universities, trying to survive by taking on an increasing number of industrial research contracts, may be undermining themselves instead by failing to charge for overhead costs in full, according to the author of a new report* on British research spending.

Peter Collins and colleagues from the Science and Engineering Policy Studies Unit (SEPSU), run by the Royal Society and the Fellowship of Engineering, have completed the first survey to identify the detailed structure of research spending in a sample of British universities, and government and industrial laboratories. Collins' stark warning on university research contract pricing policy follows a Science and Engineering Research Council (SERC) survey showing that British spending per researcher in higher education institutes lags behind that in preunification West Germany by 40 per cent.

The SEPSU study looked at research spending in 30 laboratories and university departments in 1986-87, separating salaries from all other costs (including equipment, travel, administration and laboratory maintenance). In the universities, these costs varied between 80 and 140 per cent of the salary bill. In industry, this figure rose to between 165 and 240 per cent.

According to Collins, these data show that researchers in industry are, "project for project, better equipped" than their academic counterparts. But he adds that

ASTRONOMY

Mt Graham sites cleared

Washington

ALTHOUGH its legal battles are far from over, the University of Arizona has put behind it one of the chief barriers to the construction of the controversial Mount Graham telescopes. After a three-judge panel gave the go-ahead last week, the university quickly felled trees to clear space for the first two telescopes on the sites. Over the protests of environmentalists, 24 of whom were arrested, workers began digging trenches on the sites. With just weeks left to go before the approach of winter stops further work, the construction is largely a symbolic gesture to defuse further lawsuits and to reassure the telescope's German and Vatican partners that progress is being made.

Activists plan to continue the legal battle which has already been running for three years and involved innumerable court hearings (see *Nature* 344, 479; 1990).

Christopher Anderson

the problem of maintaining a 'well found' university laboratory is compounded by the consistent failure of universities to recoup the the full indirect costs of research contracted out from industry.

The old University Grants Committee (now replaced by the Universities Funding Council, UFC) assumed that research project overhead costs are about 40 per cent of the direct costs supplied through a research council grant or industrial contract. Collins says this figure is far too low. Even so, universities on average recovered only 14 per cent of the value of external research contracts to cover overheads in 1988-89, giving the lie to recent accusations from industry that universities are overcharging (see *Nature*, 347, 216; 20 September 1990). With these external contracts increasing by nearly 14 per cent per year throughout the 1980s, Collins says universities will be "in trouble", if they do not soon start charging a proper price for their services.

Miles Hedges, the UFC's financial adviser, says the UFC is encouraging vice-chancellors to increase their research contract prices. He senses some improvement, but adds "there's an awfully long way to go". Sue Jacobs, from the industrial liaison group at Imperial College London, which has been more successful than most at recovering its overheads,

MUSEUM POLICY

Directors agree on research

London

No research, then no museum. That was the theme of an international conference on the future of scholarship in museums held last week (2 October) by the Royal Society of Arts. The message was notably spelled out by Professor Wolf-Dieter Dube, director of the State Museums of the Prussian Cultural Heritage in Berlin.

Continuing controversy over research posts at the Natural History Museum (NHM) in London spilled over into the meeting, which had been arranged long before the NHM crisis had erupted. The museum's director, Dr Neil Chalmers, contributed to a round-table discussion at the end of the meeting.

Also running through the discussion was the general feeling that museums everywhere are common property, and that their managers should maintain the expertise necessary to interpret collections. And museums must convey the essentials of new research to the public without patronizing people.

The Science Museum in London claims to follow the second precept. Popularization, said director Dr Neil Cossons, needs the support of "high-grade scholarship" to

says that (with a few exceptions) industrial companies can be persuaded to pay a fair price for university research. But she finds getting a fair deal from government departments more difficult. The problem, she says, is finding the right government official to negotiate with.

The new SERC survey is the first to calculate research spending per full-time researcher in British universities. It shows that West Germany spent £80,000 per academic researcher in 1987, compared with £48,000 in the United Kingdom. German academics are better equipped, have more support staff and are better paid than their British counterparts, the report concludes.

British spending seems to compare favourably with that in France (£36,000 per full-time university researcher). But Harry Atkinson, one of the report's authors, says that this is misleading: French research spending is biased heavily towards the laboratories of the Centre National de la Recherche Scientifique (CNRS), many of which are associated with universities.

If links between universities and CNRS laboratories are taken into account, Atkinson says, French university spending is probably similar to that in the United Kingdom.

Peter Aldhous

* The structure of research expenditure, SEPSU Policy Study 4, obtainable from SEPSU, Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

make it worthwhile. But he also noted that museums have outgrown the ability of old-fashioned academic museum managers to cope either with themselves or public demand. Victims of their own success, "museums in the past quarter-century have moved from the twilight to the spotlight". Scholars, urged Cossons, must take part in management, and not regard it as "a pollution of the purity of scholarship".

The sheer pace of change has led to confusion. Dr Klaus Sattler, a German researcher currently working at the NHM, commented that compared with the management at the NHM and its neighbour, the Victoria and Albert Museum, "the total collapse of my country in 1945 looks positively well-organized".

Responding to questions about the London NHM's corporate plan, Dr Michael Novacek, dean of science at the American Museum of Natural History, said he was disturbed at the way in which decisions on priorities had been made and that NHM staff were apparently not consulted about future research directions or warned of the dangers of "jumping on biodiversity bandwagons".

Henry Gee

SCIENTIFIC MISCONDUCT

Science meets forensic science

Washington

THE photograph below, an example of evidence from the continuing misconduct investigation of Tufts University immunologist Thereza Imanishi-Kari, shows what the detective techniques of forensic science can do to analyse laboratory research records.

Faced with the problem of dating Imanishi-Kari's records to determine if fabrication occurred in the preparation of a 1987 *Cell* paper (see *Nature* 347, 317; 1990), the staff of the congressional investigatory subcommittee of Representative John Dingell (Democrat, Michigan) focused on a series of gamma-counter tapes in the notebooks. They noted that the print quality of the tapes declined markedly as the machine's ribbon got older. On some of the tapes the staff also found a cumulative number stamp.

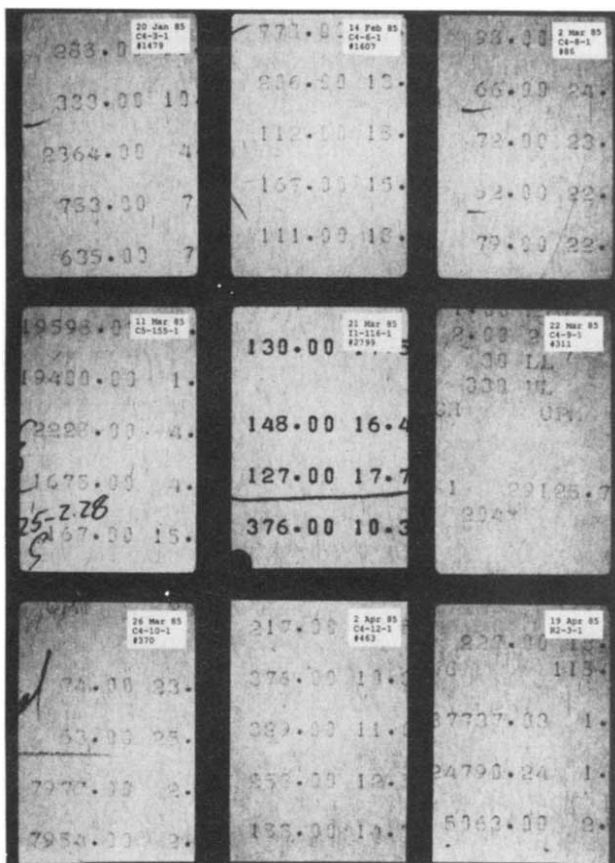
Joined by the Secret Service, the subcommittee staff decided to compare these tapes with tapes from notebooks of other researchers in the Massachusetts Institute of Technology laboratory where Imanishi-Kari had worked.

In Boston, they found that several other researchers and students had kept records of counter tapes from the 1985 period covered by Imanishi-Kari's notebooks. The investigators zoomed in on one of the tapes that came during a key part of Imanishi-Kari's experiments and, most importantly, still displayed its cumulative counter number. Around it they placed tapes from other researchers taken during the same period as the date marked in Imanishi-Kari's notebook.

When the investigators compared the tapes, as in this photograph (made available to *Nature* by the subcommittee), they immediately noticed the central one — Imanishi-Kari's tape — obviously stands out of chronological order. In that tape, the counter clearly had a new ribbon (the tape dated the next day is from a very old ribbon) and the counter number is badly out of sequence. Only one counter accessible to Imanishi-Kari had this characteristic printing style (two zeros after the decimal and so on) and font. Based on this sort of evidence, the Secret Service concluded that the date on the tape is off by many months. Whether that suggests falsification of data is, of course,

a completely different matter.

Among the other tools the Secret Service uses in such cases are hand-writing, chemical, microscopic and even neutron-activation analysis to find not only where paper came from, but when and where it was used. They can also determine, for



example, if two apparently concurrent data sets actually came from the same paper roll or notebook.

Christopher Anderson

NOBEL PRIZE

Transplantation wins again

London

IN a surprise decision, the Nobel Prize for Medicine has been awarded to Joseph E. Murray, from Brigham and Women's Hospital in Boston, and E. Donnall Thomas, from the Fred Hutchinson Cancer Research Center in Seattle, for their work on human organ and cell transplantation. Murray carried out the first successful kidney transplant between identical twins in 1954 and later pioneered the transplantation of kidneys from deceased donors and the use of whole body irradiation to induce immunosuppression.

In the late 1950s, Thomas managed to diminish the severe 'graft versus host' reaction in bone marrow transplants and showed that transplanted cells can repopulate the host bone marrow.

UK BIOTECHNOLOGY

Biotechnology no longer Wellcome

London

WELLCOME Biotechnology, since 1982 a separate division within the British pharmaceutical company Wellcome, has become the chief casualty of Wellcome's defeat by the US biotechnology company Genentech over the right to market tissue plasminogen activator (TPA) in the United States (see *Nature* 344, 692; 1990).

Salvador Moncada, director of research for the Wellcome Foundation, says that the loss of the valuable US market for Wellcome's TPA, together with the cessation of contracts to manufacture animal vaccines in 1992, means that the biotechnology division has lost its economic *raison d'être*. It will be wound up over the next few years.

The remaining products from Wellcome Biotechnology (Wellferon, an alpha-interferon, and Campath, an antibody-based anti-cancer agent) and some biotechnology research will be integrated into other sections of the company. Moncada says that the restructuring will involve a reduction in the "head count" of researchers, but he says that the company is looking for ways to retain as many as possible of the division's 100 or so researchers. Decisions on which research projects are to be retained will be taken in the coming weeks.

The closure of Wellcome Biotechnology is part of a restructuring that includes Wellcome's withdrawal from human vaccine production. News of this has generated considerable criticism, as Wellcome is the single remaining large-scale producer of vaccines in the United Kingdom, and an important supplier for the National Health Service (NHS). But the company says that production will continue until the NHS has found alternative suppliers.

Peter Aldhous

The award is the third in the field of transplantation in the past 30 years, following Sir Peter Medawar and Sir MacFarlane Burnet's 1960 prize for discovering immunological tolerance and the 1980 award to Jean Dausset, George Snell and Baruj Benacerraf for the discovery of HLA human histocompatibility antigens. This year's prize is controversial, as it can be argued that other researchers working at about the same time made equally important contributions to the development of transplantation as a clinical technique, notably two Frenchmen, Jean Hamburger and Georges Matté. Hans Wigzell, chairman of the Nobel Committee, says it is "more difficult in the clinical world [than in basic science] to identify pioneers".

Peter Aldhous

Sellafield under scrutiny

London

THE UK Health and Safety Executive (HSE) plans to extend the survey which earlier this year suggested a link between the high incidence of leukaemia in children in the West Cumbrian village of Seascale and their fathers' exposure to radiation at the Sellafield (formerly Windscale) nuclear reprocessing plant (see *Nature* **343**, 679; 1990).

The new HSE study, which will cover everybody who has worked at Sellafield since 1949, is one of a number of projects designed to investigate the controversial link between paternal radiation exposure and childhood cancer originally suggested by Professor Martin Gardner of the University of Southampton. These studies should soon begin, once official clearances have been obtained and problems with data confidentiality resolved.

The HSE study will compare the full work histories of the fathers of leukaemic and unaffected children, looking at other factors besides radiation dosages alone. Exposure to carcinogenic chemicals, for example, has been put forward as an alternative explanation for Gardner's results. Any Sellafield workers wishing to have their records excluded from the study must get in touch with the HSE by the end of November.

Gerald Draper, from the University of Oxford, plans a wider study, linking Oxford's National Registry of Childhood Tumours with the National Radiological Protection Board (NRPB)'s radiation dose records for all British radiation workers. But Draper says progress has been "snarled up" because of the confidentiality of vital data held by the Office of Population Censuses and Surveys (OPCS).

To link the two databases, Draper needs to know the date of birth of the fathers of leukaemic children, to check that matches between the names of fathers and workers on the NRPB database are genuine. A 1938 act of parliament allows only OPCS staff access to these data.

Draper says a compromise should be possible, with OPCS staff doing some preliminary analysis and providing the Oxford team with summary data, so that individuals cannot be identified. He now hopes to get approval for the study within the next few months.

If the 'Gardner effect' is real, and the risk factor is anything like as large as Gardner suggested (a seven to eightfold increase in the chance of fathering a leukaemic child for fathers receiving a total radiation dose of 100 milliSieverts, or more than 10 mSv in the six months before conception), Draper says his survey should be large enough to detect the effect.

In Scotland, where population records

are subject to a different law, data confidentiality has not been a big problem. But studies have still been held up by the need to get official approval from the HSE and the Department of Health.

The Committee on Medical Aspects of Radiation in the Environment, independent scientific advisers to the UK government, said that further study following the



Gardner report should be co-ordinated centrally to avoid unnecessary intrusion into the lives of British radiation workers (see *Nature* **344**, 576; 1990). This was in-

RESEARCH IN JAPAN

Rent-a-lab welcome for foreigners

Tokyo

JAPAN'S Ministry of International Trade and Industry (MITI) is opening up some of its major research programmes to foreign participation and making some of its best-equipped facilities available for rent in a campaign designed to counter foreign complaints that the Japanese research system is closed to outsiders.

At the end of last month, MITI announced that three huge programmes run by the New Energy and Industrial Technology Development Organization (NEDO) are now open to foreign participation. The programmes are in advanced chemical processing technology, software development and in the design of clothes that "reflect human sensibility". But the change in policy is unlikely to bring in a horde of foreign companies as it was announced only in the *MITI Gazette* and scarcely a month remains for applications to be filed.

More foreign interest may be generated from a second MITI decision, announced last week, to open some facilities for public use. For as little as ¥200,000 (\$1,460) foreign companies or individuals will be able to rent a ten-second free-fall ride aboard a

tended to avoid duplication of intrusive studies requiring questionnaire data, but has also held back those simply involving the analysis of existing records.

Leo Kinlen, from the Cancer Research Campaign's laboratories in Edinburgh, last week received approval for a study that will link records of Scottish leukaemia cases and controls with radiation records for the entire Scottish nuclear industry. He hopes to complete arrangements for access to the industry's radiation dose records within the next few weeks. A second study is planned by the Scottish Health Service, but both Scottish surveys may be too small to give a definitive answer on the existence of the Gardner effect. Nevertheless, Kinlen says the studies should give an indication of the "size of the problem [in Scotland], if problem there is".

Two surveys commissioned to investigate the smaller excesses in leukaemia incidence near nuclear sites at Dounreay in Scotland, and Aldermaston and Burghfield in southern England, are now nearing completion. The Dounreay study should be published within the next few months, the Aldermaston and Burghfield study before the end of 1991.

Peter Aldhous

■ Researchers from AEA Technology show that plutonium-239 does not accumulate in large quantities in human testes. This has been put forward as a mechanism to explain the Gardner effect. See Scientific Correspondence page 521.

microgravity capsule that can be shot down a disused coal mine shaft in Hokkaido. The mine shaft at the Japan Microgravity Center is 710 metres deep and MITI claims that it provides longer near-zero-gravity conditions than at any other ground-based facility.

Also available for rent will be some of the world's most powerful ion beams, courtesy of the new Ion Engineering Center in Kansai science city, and the facilities of the Research Center for Industrial Utilization of Marine Organisms, the Japan Ultrahigh-Temperature Material Research Center and the Applied Laser Engineering Center.

All these institutes are run as corporations by NEDO which was reorganized in 1988 to absorb most of MITI's major research and development projects. The corporations receive half of their funding in the form of investment from NEDO, private companies and local government. Rental charges are intended to cover operating costs and not to provide a profit, says MITI. At first, the major users are expected to be the Japanese companies that have already invested in the institutes.

David Swinbanks

Early case of AIDS in the USA

SIR—Two recent news items in *Nature*^{1,2} highlight the case history of a 25-year-old seaman who died in Manchester, England, of *Pneumocystis carinii*, and in retrospect, of human immunodeficiency virus (HIV) infection, in 1959, the same year as the earliest serologically confirmed case of AIDS in Africa. The polymerase chain reaction (PCR) was used to amplify HIV genetic material in stored tissue specimens, although distinction between HIV-1 and HIV-2 was inconclusive³. Contrary to a statement in one of the *Nature* articles¹, it is not known whether the Manchester seaman spread

help clarify the epidemiology and viral evolution leading to the AIDS pandemic.

ROBERT F. GARRY

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1. Opinion *Nature* **346**, 92 (1990).
2. Concar, D. *Nature* **346**, 95 (1990).
3. Corbett, G. et al., *Lancet* **336**, 51 (1990).
4. Stretton, T. *Nature* **346**, 505 (1990).
5. Frøland, S.S. et al., *Lancet* **i**, 1344–1345 (1988).
6. Elvin-Lewis, M. et al., *Lymphology* **6**, 113–121 (1973).
7. Witte, M.H. et al., *J. Am. med. Soc.* **251**, 2657 (1984).
8. Garry, R.F. et al., *J. Am. med. Soc.* **260**, 2085–2087 (1988).
9. Mullis, K. & Faloona, F. *Meth. Enzym.* **155**, 335–350 (1987).

Documentation of an AIDS Virus Infection in the United States in 1968

Robert F. Garry, PhD; Marlys H. Witte, MD; A. Arthur Gottlieb, MD;
Memory Elvin-Lewis, PhD; Marise S. Gottlieb, MD; Charles L. Witte, MD;
Steve S. Alexander, PhD; William R. Cole, MD; William L. Drake, Jr, MD

The acquired immunodeficiency syndrome was first recognized as a clinical entity in the United States in the early 1980s; however, the issue of when human immunodeficiency virus, the causative agent of the acquired immunodeficiency syndrome, was introduced into at-risk populations in the United States is unresolved. Previously we reported the case study of a 15-year-old male patient admitted to St Louis City Hospital in 1968 for extensive lymphedema of the penis, scrotum, and lower extremities. He had no history of travel outside of the Midwest, intravenous drug abuse, or blood transfusion.

St Louis City Hospital in 1968 for extensive lymphedema of the penis, scrotum, and lower extremities. He had no history of travel outside of the Midwest, intravenous drug abuse, or blood transfusion.

his HIV infection⁴. However, the other commentary² refers to another report of serologically confirmed HIV-1 infection in a Norwegian family including a father (the index patient, also a seaman, whose multisystem illness began in 1966), his wife, and daughter, all of whom died in 1976⁵.

Antecedent to these reports, we documented HIV-1 infection in a 15-year-old black male admitted to St Louis City Hospital in 1968 with extensive genital and bilateral lymphoedema and systemic chlamydial infection, who died less than a year later with a progressive cachexia and disseminated Kaposi's sarcoma^{6–8}. In contrast to the two well-travelled sailors cited above, our patient had never ventured outside the St Louis area. Western blot analysis of frozen serum and antigen capture enzyme-linked immunosorbent assay of stored tissues confirmed infection with HIV-1⁸. Proviral DNA has recently been detected in his tissues by PCR⁹ in collaboration with J. Sninsky and S. Kwok (Cetus Corporation, Emeryville, California) but nucleotide sequence analysis is not yet complete.

Rather than representing the initial incursions of HIV into Britain, Norway and the United States, these documented AIDS patients from the pre-AIDS era support the contention that sporadic cases occurred in developed countries at least several decades before AIDS was formally recognized in the United States in the early 1980s. Sequence analysis of proviral DNA from these patients should be of interest to elucidate relationships to current HIV-1 and HIV-2 strains and thereby

Science sells its soul

SIR—There is a crisis in science, particularly in the biomedical field and particularly in the United States, resulting from a new and spreading perception of the scientific endeavour. The profession of science has shifted from its basic underpinnings. It used to be, and still is in some degree, that there was an allure, a challenge, an adventure of the mind in the solution of riddles. Many were willing to work long hours, even with little pay, to make a discovery and to be recognized for the discovery.

But this has all been changed, particularly in the past decade, the 'me too' decade, for added on has been the allure of money. The thought of many established investigators has become "why should others gain large profits from my work; why should I not also cash in on it?" So there has evolved a scramble to make arrangements, to seek deals, to make sure that one will still enjoy scientific work while raking in the cash. Postdoctoral fellows and students cannot fail to notice this behaviour of their mentors, and to be influenced by it. The adventure is still sweet, but a monetary component has been added.

It used to be that a scientist worked in a university or medical school to achieve the goals of the institution — teaching and research. Now he or she is asked or coaxed to perform another function, that of bringing in money to the university. If

there are no arrangements with industry to bring in patent-generated money, or licensing fees, one is looked upon as an outcast, not doing one's share to keep the university viable.

Thus, increasingly, the scientific endeavour is being looked upon by young scientists as a way of making money while gaining scientific acclaim on the side. Companies have been set up by scientists to rake in the cash; the supposed rationale has been to bring desirable products onto the public market, a public benefit as a product of the research. But to other scientists, old or young, still imbued with the former ideals of science, all this is hypocrisy. Although they recognize that science cannot be amoral, that 'pure' science is a fiction, that it is influenced by the many political and social forces in our culture, they abhor the commercialization of science.

The future of scientific research is in danger not only from a decline in funding, but also from within. As idealism drains away so too does the spirit that is the adventure of scientific research, drawing to it less and less of our dedicated youth. Sinclair Lewis's *Arrowsmith* is out; biotechnology companies are in.

PHILIP SIEKEVITZ

Rockefeller University,
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New York 10021-6399, USA

Missing the joke

SIR—I would like to make a minor but important correction to an item that appeared in the Opinion section (*Nature* **346**, 398; 1990). In connection with the use of the same vehicle for carrying food and garbage, US General Accounting Office wags are quoted as quipping: "What has four wheels and fries?" Here in America the way the joke runs as I know it is: what has four wheels and *flies*?

GEORGE CRISCIONE

Communications of the ACM,
11 West 42nd Street,
New York, New York 10036, USA

Celebrating Charles Darwin

SIR—It has recently come to light that Britain has fewer public holidays than many other countries. Your readers may wish to know that we have formed a Forum to Instate a Charles Darwin Day. It seems to us fitting that Britain should have a regular public holiday to commemorate the man who first explained humanity to humanity.

Further information may be had from the undersigned.

K. R. HARRISON

7 Birchfield Close,
Liverpool L7 9LZ, UK

Which way now for Europe?

SIR—Your leading article (*Nature* 346, 203; 1990) deriding the indiscretions of Mr Nicholas Ridley quite missed the point of what he was saying. He was not saying, as you impute, that “rigid genetic determination” assures German domination. The view that he was expressing, and which is shared by many other people (perhaps not such a minority as the disclaimers of his ideas have hastened to imply), is that in the German position lies the seat of forces which, in my lifetime, have generated three major struggles to dominate Europe. The third is clearly in progress.

Those forces are associated with its geography, compactness, social attitudes and practices, natural resource limitations, possibly a different genetic mix from that of its neighbours — I know of no “respectable biologist” who would claim it to be identical — and many other factors, some traditional, some imposed afresh on each new generation by its uniquely landlocked situation. They have not only pushed the German people into two world wars this century, but have also inspired them to behave in ways so far beyond the pale of civilized human conduct that they have outraged the whole world — a triviality that you ignore. The partition of Germany in 1945 and the emergence of two

superpowers, with the (perhaps only) common objective of suppressing a recurrence of a German-led domination, has proved to be only partly efficacious in achieving this objective.

What many fear is that these forces, which have inspired the Germans to such superior economic performance, even within these postwar constraints, will, now that partition is ended, Germany unified and the superpowers down to a single, far-distant one, move the German nation to use other forms of domination in the course of time if political and economic domination prove inadequate for the German appetite. I know of no reason why a unified Germany of AD 2000 should have feelings, ideas, aspirations, hopes, resentments and fears different from a unified Germany of AD 1900 or 1930 — or why it should act differently. That the Germans have so far been contained to the expression of economic domination is no guarantee that, now free from this containment, they will be content to act as though the constraints of partition and the Warsaw pact were still in place and NATO obligations binding.

W. FRANK HARRIS

Acomb High House,
Acomb, Hexham,
Northumberland NE46 4PH, UK

To go or not to go

SIR—When invited to visit colleagues in Beijing, I too asked myself (*Nature* 346, 594; 1990) whether my visiting China would be perceived as implicit support for its present government. After ascertaining that the American Physical Society had no clear policy on individual visits to China (as opposed to attendance at officially organized conferences), I asked several of my colleagues from the People's Republic of China. Remarkably, the response to my question “Should I go?” was unanimous: all felt that individual visits, so long as they were not government-sponsored, could only benefit scientists working in China, and I was actually encouraged to make my trip. The same response was given by this university's Chinese Student and Scholar Association, which is not known for pro-Li sentiment, and which presumably is immune to any bias that might inhibit a colleague from discouraging the travel plans of another.

Ironically, after obtaining this ‘clearance’ and my visa, I was unable to make the trip. However, in the absence of new data or arguments, I would not hesitate to visit colleagues there in the future.

MICHAEL H. SALAMON

Physics Department,
University of Utah,
Salt Lake City, Utah 84112, USA

SIR—By referring to Mr Nicholas Ridley's recent *Spectator* interview in which he revealed his concern about German characteristics, as an “outburst”, you accuse him of being intemperate.

But this is not the main substance of your complaint. You have inferred that his concern about German characteristics indicates his belief in their genetic determination. You go on to express outrage that such a view could be held by grown-up people against the beliefs of respectable biologists.

First, this response is as intemperate in tone as were Ridley's original utterances, betraying a depth of feeling inappropriate to the dispassionate and disinterested posture of a scientific journal of *Nature*'s standing. Second, there is no reason to believe that Ridley believes that German characteristics are necessarily genetic in origin. Indeed, later in your article, you allow that such characteristics as can be determined (in so far as Ridley apparently fears them) are “recognizably the products of social institutions”.

Whether the product of social institutions or inherited, Ridley may argue, these characteristics are equally to be feared.

It seems that you have used the Ridley “outburst” as an opportunity to express a dogmatic position in the ‘nature versus nurture’ argument — which argument is

clearly not relevant to Ridley's fears. Thus, far from demonstrating the “primitive roots” of Ridley's views, you have used the primitive methods of guilt-by-association in aligning Ridley's views with those who believe in the influence of genetics on behaviour.

C. G. Jung's views concerning the behaviour of Germans and other groups remain deeply unpopular in some quarters as they appeared to fuel the Nazi obsession with race — in Jung's case also there is no reason to believe that he considered *inheritance* to be the mode of passing national characteristics from generation to generation.

No, the legitimate fear of identifying national or racial characteristics is that this can lead to discrimination against vulnerable minority groups. This fear should not be confused with the fear of the uprising of a traditionally assertive nation.

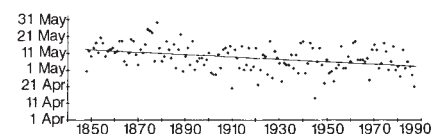
If there remains doubt concerning the pedigree of the perception by non-Germans of German characteristics, further reference should be made to Tacitus, whose description of German characteristics in *Germania* is very similar to the observations of some more modern commentators of Ridley's persuasion.

J. F. MACKENZIE

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Ice-out in Maine

SIR—The folks up in northern Maine have kept an accurate record of the time of ice-out on their largest lake, Moosehead Lake, for the past 142 years. In the spring, ice-out comes suddenly, usually in one day. Moosehead Lake was the centre of the logging industry in the late nineteenth and early twentieth century, and ice-out in the spring meant fresh supplies by steamboat to the isolated logging camps on the Northeast Carry. So ice-out, like Christmas, was celebrated, remembered and luckily recorded. More recently, ice-out contests have been sponsored by the Moosehead Lake Region Chamber of Commerce. Thus, an exact record of the date of this event each year since 1848 has been preserved. When the dates are plotted and the best fit line drawn, it appears that on average ice-out occurs



about 10 days earlier now than in 1848. Perhaps these data will be of interest to those studying climate change, but up here in Maine we are going to take them seriously and start reducing greenhouse gases.

DONALD G. COMB

Frost Pond,
Maine, USA

Pulsar seen in SN1987A remnant?

Optical pulsations may have been detected from the region of the Magellanic Cloud supernova SN1987A by astronomers in Brazil. If confirmed, the observations probably presage pulsed X-ray emission.

THE announcement on 3 October, on astronomers' worldwide electronic mail networks, of the discovery of 18.4 millisecond optical pulsations in the Magellanic Cloud supernova, SN1987A, may be one of the missing pieces in a jigsaw not yet complete. The announcement, by astronomers in São Paulo, Brazil, came just after a meeting on Elba on the supernova* devoted in part to explaining why pulsations had not yet been seen. Astronomers from the European Southern Observatory had shown a long list of upper limits, demonstrating the fruitlessness thus far of their search for optical pulsations. The Brazilian detection shows that luck, too, is an essential ingredient of discovery.

The Brazilian astronomers, F. Jablonski, R. Baptista, C. D. Gneiding, J. E. Steiner and F. Elizalde, claim the detection with the 1.6 metre telescope of the Laboratório Nacional de Astrofísica in southeast Brazil, of quasiperiodic oscillations with centroid frequencies near 54.5 Hz (a period of 18.3 milliseconds) and bandwidth 0.05 Hz: the oscillations were first detected on 17 September, the day on which the Elba conference began, and again on 28 September.

The Brazilian group interprets the 18.3-millisecond optical signal as reprocessing of the beam from the rotating neutron star formed in SN1987A. The pulsations would be at a well-defined frequency if the Earth were in the sweep of the emission beam of the pulsar as it rotates, because the rotation of a neutron star is very stable. The pulsations seen in Brazil were not of this nature; their frequency changed noticeably from hour to hour.

That is why the Brazilian astronomers propose that the neutron star is oriented pole-on towards the Earth so that we see the flashes not directly but indirectly, reflected from something nearby — infalling or orbiting clouds, or, less plausibly, a companion star. The result is therefore a train of pulses Doppler-shifted by reflection in moving mirrors.

These observations remain to be confirmed. (Previously reported 2 kHz pulsations are now known to be due to inductive pick-up in the equipment but, while 54 Hz is curiously near mains frequency, the Brazilians list an impressive set of null observations of stars other than the supernova.) But confirmation will provide

the second piece of evidence, and the long-awaited confirmation, that SN1987A produced a neutron star when it exploded on 23 February 1987. The first evidence was, of course, the neutrino emission then seen by the Kamiokande neutrino detector in Gifu, Japan, and its sister detector, known as IMB, in Ohio. Since then no further trace of the neutron star had until now been seen.

The total ultraviolet, optical and infrared emission from the supernova is about 500 times more than the pulsed emission and arises from reprocessed energy from the radioactive decay of cobalt-56, itself produced from radioactive nickel-56 formed in the explosion. The light curve of the supernova (the evolution of its brightness) is still, after three years, accurately tracking the exponential decay of radioactive cobalt, uncontaminated by evidence even of longer-lived isotopes such as cobalt-57 and titanium-44, expected to have been formed with nickel-56.

The most serious persisting doubt about the existence of a neutron star in SN1987A is the lack, for two years from August 1987, of the X-rays expected by degradation of the γ -rays emitted by cobalt-56. SN1987A has now faded from the view of all active X-ray telescopes. It is not now seen during the monthly inspections by the Japanese GINGA satellite and was not seen in the brief glance on 23 May this year by the Soviet GRANAT instrument. This last of these limits was at a level of just a few thousandths of the luminosity of the Crab Nebula, itself powered by an ageing 950-year-old pulsar which would be expected to be much less powerful than a lusty three-year-old.

The strongest upper limit on the X-ray luminosity is from a test observation of the Magellanic Cloud during the so-called performance verification phase of the West German X-ray telescope on the ROSAT satellite. The supernova was not seen above a luminosity of 5×10^{34} erg s⁻¹.

X-ray emission from a neutron star is expected when material falls on to it and releases gravitational potential energy. The material can be drawn in onto a newly formed neutron star: it can be driven back from the expanding ejecta by a reverse shock in the outflowing material and sucked in (according to a suggestion originally made by Stirling Colgate, Los Alamos National Laboratory) by the cooling of the neutron star's surface by continuing

neutrino emission. But, however much material there is (within limits), the luminosity of the radiation is pushed up to 3×10^{38} erg s⁻¹ by a self-limiting process — if too much material falls in, so that the luminosity rises above this limit, radiation pressure expels the excess.

The infalling material, if there is any, quenches pulsations from the neutron star, so that the gravitational potential energy may at first show up as steady optical emission from the kind of atmosphere that forms on an isolated neutron star (R. Chevalier, University of Virginia). The atmosphere is unstable and dissipates on a timescale of about a year, ultimately revealing emission from the neutron star itself. Perhaps that is why the optical pulsations have only now become visible.

The present lack of unpulsed X-ray emission shows that the region around the neutron star is now very clean, with no material there to fall back. That is borne out by the discovery in August that the supernova has turned on again in radio emission (it was first seen at radio frequencies soon after the explosion in 1987, but the radio power quickly died away). Australian radioastronomers at the Molongolo Observatory Synthesis Telescope (immodestly known as the MOST) have recently seen the supernova progressively increasing in brightness at levels upwards of 1 millijansky. At the Elba conference, it was calculated that the radio flux was then increasing as $t^{6.9}$, measured since the 1987 explosion — a step-function switch-on like a window opening.

There was speculation at Elba about the explanation of this phenomenon. Either the pulsar is revealing itself or some of the expanding ejected body of the supernova has hit a lump of circumstellar material. If the former, the appearance of the radio emission would be evidence that the line of sight has cleared of material, letting the radio emission out. Intercontinental aperture synthesis techniques could distinguish the two possibilities; if the radio signal were to coincide with the supernova, then that is probably the pulsar; if the radio source is displaced by a fraction of an arcsecond, then it probably comes from the ejecta instead. But what is needed first of all is confirmation of the Brazilian result. Then, if there really is pulsed optical emission, the detection of pulsed X-ray emission is likely soon to follow.

Paul Murdin

*Workshop on SN1987A and other supernovae, 17-22 September, Marciana Marina, Elba.

Flocks of African fishes

John C. Avise

MORPHOLOGICAL and molecular evolution can march to the beats of different drummers. Thus horseshoe crabs, 'living fossils' that have changed little in morphology over tens of millions of years, exhibit 'normal' patterns of genetic variation and divergence in proteins and DNA^{1,2}, whereas humans and chimps, which differ so obviously in anatomy and way of life, are more than 99 per cent identical in polypeptide sequences³. Even against such examples, the apparent decoupling of morphological and molecular evolution reported for African rift-valley lake fishes on page 550 of this issue is stunning. Meyer *et al.*⁴ report that 14 assayed species, representing nine genera of cichlids endemic to Lake Victoria, show almost no differentiation at some 800 nucleotide positions in the cytochrome *b* gene, two transfer RNA genes and the normally highly variable portion of the control region of mitochondrial (mt) DNA. The mean level of mtDNA sequence divergence among these species and genera is less than that within a single species of horseshoe crab², or within the human species, which itself exhibits low intra-specific mtDNA differentiation compared to many vertebrates, including other fishes.

The cichlid species flocks in Lake Victoria and other African rift lakes have long intrigued biologists as prime examples of 'explosive evolution'⁵. Lake Victoria is less than one million years old, yet it contains some 200 species of cichlids, almost all of which are endemic. This assemblage exhibits moderate diversity in external morphology (see figure), but far more striking are the ecological and trophic specializations represented^{6,7}. There are algae grazers, plankton and detritus feeders, pharyngeal snail crushers, and insect and fish predators. Some species are paedophages, eating fish embryos by engulfing the snout of a mouth-brooding female and forcing her to jettison the brood. One species feeds on scales rasped from tail fins, while another (in Lake Malawi) plucks the eyes from other fishes. The discovery of a near lack of mtDNA differentiation among representative Lake Victoria cichlids implies that this remarkable kind of organismal diversity evolved very recently (within the past 200,000 years), probably from a single common ancestor within the lake.

The hypothesis of recent monophyly for the Lake Victoria flock is further supported

by mtDNA comparisons involving other African cichlids⁴. For example, assayed species in nearby Lake Malawi differed from those in Lake Victoria by more than 50 base substitutions, whereas the Lake

described species (over 500 of them) were placed in a single genus, *Haplochromis*, but in recent years a morphological reanalysis based on hennigian principles prompted a taxonomic splitting into a large number of genera and subgenera⁷. Many of these taxa have representatives in at least four of the African great lakes, such that "the overall picture is one of a super-flock comprised of several lineages

whose members cut across the boundaries imposed by the present-day lake shores"⁸. The molecular findings of Meyer *et al.*⁴ stand in stark opposition to this concept. If the mtDNA results are corroborated and extended to many additional haplochromine species, yet another taxonomic realignment will be in order, one in which the lineages recognized would coincide almost perfectly with the present-day lake-shore boundaries.

The phylogenetic findings with mtDNA set the stage for further research on the ecology and genetics of African great-lake fishes. Did the intra-lacustrine speciations take place sympatrically, or did they occur at times of geomorphological subdivision of a lake basin? Is there a simple genetic basis to the ecological and morphological shifts, and are particular morpho-genes involved? Could at least some of the morphological shifts represent phenotypic or ontogenetic plasticity within species, as has been suggested for trophic polymorphisms in some New World cichlid fishes⁹? (Additional assays of nuclear genes are needed to decide whether the gene pools of all morpho-species are truly distinct.) Is there some peculiarity in the genetic makeup, behaviour or ecology of cichlid fishes that predisposes them to explosive evolutionary radiation, whereas other fish taxa in these lakes appear to evolve at normal rates? What are the ecological and behavioural ramifications of habitat

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Examples of cichlid species from Lake Victoria assayed by Meyer *et al.*⁴. Top, *Ptyochromis sauvagei*; middle, *Lipochromis obesus*; bottom, *Astatotilapia piceatus*. (Reproduced from ref. 7.)

Victoria cichlids differed from one another by an average of only three mtDNA mutations. Among 20 members surveyed of the 200 or so endemic cichlids in Lake Malawi, two distinct mtDNA lineages were observed, but these remained much more closely related to one another than to any non-Malawi cichlids examined. Thus the molecular findings appear to eliminate an alternative picture for the Lake Victoria and Lake Malawi species flocks — that they might represent the polyphyletic products of numerous invasions by taxa that had become highly differentiated before each lake's formation.

The phylogeny and taxonomy of the African cichlid flocks have long been under dispute. Traditionally, most of the

partitioning by so many closely related forms? These are all obvious but difficult questions. The findings of Meyer *et al.*⁴ at least show that there really is a phen-

1. Selander, R.K., Yang, S.Y., Lewontin, R.C. & Johnson, W.E. *Evolution* **24**, 402–414 (1970).
2. Saunders, N.C., Kessler, L.G. & Avise, J.C. *Genetics* **112**, 613–627 (1986).
3. King, M.C. & Wilson, A.C. *Science* **188**, 107–116 (1975).
4. Meyer, A., Kocher, T.D., Basasibwaki, P. & Wilson, A.C. *Nature* **347**, 550–553 (1990).
5. Mayr, E. *Evolution and the Diversity of Life* (Harvard Univ. Press, 1976).
6. Fryer, G. & Iles, T.D. *The Cichlid Fishes of the Great Lakes of Africa* (TFH, Neptune City, New Jersey, 1972).
7. Greenwood, P.H. *The Haplochromine Fishes of the East African Lakes* (Cornell Univ. Press, 1981).
8. Greenwood, P.H. *Bull. Br. Mus. nat. Hist. (Zool.)* **39**, 1–101 (1980).
9. Sage, R.D. & Selander, R.K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4669–4673 (1975).
10. Echelle, A.A. & Kornfield, I. (eds) *Evolution of Fish Species Flocks* (Univ. Maine Press, Orono, 1984).

omental pattern of within-lake evolutionary radiation to be understood.

The cichlids of the great African lakes are the most spectacular of a small number of fish species flocks in drainage basins scattered around the world¹⁰. Most are threatened with extinction from anthropogenic causes, and indeed the cyprinid flock in the Philippines' Lake Lanao has already been lost. The African cichlids are also in rapid decline, primarily because of the introduction of exotic predatory

fish. The great African rift lakes and their faunas will someday disappear as tectonic plate movements continue to split the continent. Premature closure of this extraordinary evolutionary theatre and play through human activities would be a biological tragedy of the first order. □

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ASTROPHYSICS

X-ray burster is theory buster

Michael Garcia

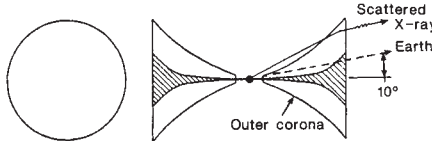
THE hallways at the most recent meeting of X-ray astronomers (held in Bologna in September) were buzzing with rumours about the latest results from Japan's GINGA X-ray astronomy satellite. One of the more spectacular rumours was that the satellite had detected an X-ray burst from the bright X-ray source (AC211) in the centre of the globular cluster M15. Not only was this the first detection of an X-ray burst from this object, but the rumour was that it was a truly spectacular burst — unusually bright and long, and containing features rarely (if ever) seen in X-ray bursts. On page 534 of this issue¹, Dotani *et al.* reveal that the burst was every bit as spectacular as rumoured.

X-ray bursts are commonly seen from low-mass X-ray binaries (LMXBs), which are short-period binaries (orbital periods of a week or less) containing a neutron star or black hole which accretes matter from a companion star. The bursts occur when sufficient matter has accreted on to the neutron star to fuel a runaway thermonuclear reaction. Once started, the entire surface of the neutron star ignites within about a second, and it burns for a few hundred seconds. LMXBs are seen throughout our Galaxy, but are concentrated in globular clusters which have stellar densities sufficient to allow the formation of very close binaries through near collisions between stars.

AC211 is unique among the globular-cluster LMXBs because it is the only one for which an optical counterpart has been found. Optical studies have allowed astronomers to determine its structure to greater precision than any other cluster LMXB. For example, we know the orbital period is 8.5 hours, and that the optical light is strongly modulated at this period². The heliocentric radial velocity of the system is also modulated at this period, but the mean velocity is offset from the host cluster's by about 150 km s⁻¹. This remarkable fact was initially interpreted³ to mean that the binary was being thrown out of the globular cluster by the same stellar collisions as formed it. Subsequent

studies have shown that the lines with these high radial velocities are formed in a wind which flows out from the system, and that AC211 itself is not being ejected from the cluster⁴.

The strong optical modulation suggests that the binary system is viewed nearly edge-on, as this geometry enhances the observed modulation caused by two stars orbiting each other. The ratio of the X-ray



This schematic diagram of an accretion-disk corona source shows how the accretion disk (shaded) can block our view of the central neutron star, therefore lowering the apparent X-ray luminosity of the LMXB. The dense neutron star is on the right and its companion, on the left. (From ref. 5.)

to optical luminosity (L_X/L_{opt}) is much lower than typical⁵ and has also been taken as evidence that the system is viewed nearly edge on, and that the accretion disk blocks our view of the central neutron star, therefore reducing the apparent X-ray luminosity. The X-rays we do see are presumed to be scattered off an accretion-disk corona (ADC) surrounding the central source, and extending above the accretion disk⁶ (see figure). There are at least five other LMXBs that show evidence of such a corona⁶, so before the announcement of the GINGA burst this model of AC211 was reasonably well accepted.

However, the ADC model implies that even during an X-ray burst we should see only a fraction of the total X-ray emission, and this indeed what was seen⁷ during a burst from another ADC source, 4U2129+47. At the peak of the burst from AC211 the X-ray flux reached about twice the Eddington limit — the theoretical limit which the flux cannot exceed. The violation of this limit is in itself disturbing, but may be at least partially explained if the matter being accreted in AC211 is

primordial and therefore devoid of any elements heavier than helium (atomic absorption contributes to the limit). But the idea that we are only seeing a fraction of the total X-rays when we see twice the theoretical limit is clearly untenable, so that the idea that the accretion disk blocks our view of the central neutron star must be wrong. The only way to salvage it is to assume that the disk changes shape during the burst, or that the burst was from another (previously unknown) source within a few arcseconds of AC211.

Rather than invoke such *ad hoc* explanations, it seems more reasonable to attribute the low ratio L_X/L_{opt} to an unusually high optical luminosity, as suggested by Fabian *et al.*⁸. This idea seemed an unnecessary complication when first put forward, but with the discovery of this bright burst it now seems to have been remarkably prescient. The known lack of heavy elements in the globular cluster M15 (and presumably in AC211 as well) is conducive to forming an optically bright accretion-disk corona. Fabian *et al.* point out that the physical conditions which allow an optically bright accretion-disk corona would also allow a rather large outflow of material off the surface of the disk. This material may be exactly that seen leaving the system at a few hundred kilometres per second^{4,9}. The rate of mass loss in this wind is high enough to cause accelerated and unstable evolution of the binary system.

This evolution could ultimately cause the orbital period of AC211 to be drastically decreased. Interestingly, the only other cluster LMXB for which the orbital period is known is 4U1820-30, in the cluster NGC6624, which has the shortest orbital period of any known binary — 11 minutes (ref. 10). Bailyn and Grindlay¹¹ have suggested that AC211 will evolve into a system like 4U1820-30; the rapid and unstable evolution implied by the idea proposed by Fabian *et al.* seems to support this suggestion. □

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1. Dotani, T. *et al.* *Nature* **347**, 534–536 (1990).
2. Ilovaisky, S.A., Auriere, M., Chevalier, C., Koch-Miramond, L., Cordini, J.-P. & Angebault, L.P. *Astrophys. J.* **179**, L1–L4 (1987).
3. Naylor, T., Charles, P.A., Drew, J.E. & Hassall, B.J.M. *Mon. Not. R. astr. Soc.* **233**, 285–304 (1988).
4. Ilovaisky, S.A. *ESA Spec. Pap. SP296*, 145–150 (1989).
5. McClintock, J.E., London, R.A., Bond, H.E. & Grauer, A.D. *Astrophys. J.* **258**, 245–253 (1982).
6. Mason, K.O. in *Physics of Accretion onto Compact Objects* (eds Mason, K.O., Watson, M.G. & White, N.E.) 29–57 (Springer, Heidelberg, 1986).
7. Garcia, M.R. & Grindlay, J.E. *Astrophys. J.* **313**, L59–L64 (1987).
8. Fabian, A.C., Guilbert, P.W. & Callanan, P.J. *Mon. Not. R. astr. Soc.* **225**, 29p–31p (1987).
9. Charles, P.A. *ESA Spec. Pap. SP296*, 129–137 (1989).
10. Stella, L., Friedhorsky, W. & White, N.E. *Astrophys. J.* **312**, L17–L21 (1987).
11. Bailyn, C.D. & Grindlay, J.E. *Astrophys. J.* **316**, L25–L29 (1987).

J. S. Bell (1928–1990)

JOHN Stewart Bell, who died unexpectedly on 1 October, became famous for his work on quantum mechanics, but he also made many major contributions to accelerator physics — many-body theory — especially as applied to nuclei–quantum field theory and particle physics. His successful prediction that neutrinos would suffer nuclear ‘shadowing’, despite having a mean free path in matter of millions of miles, is just one example of his many ‘conventional’ contributions.

His work on the ‘Alder–Bell–Jackiw’ anomaly, which showed that certain symmetries of classical theories cannot be maintained in quantum theories, led not only to important constraints on models of elementary particles, but also to some very surprising and deep connections between physics and geometry which are still being explored.

Bell’s first contribution to what he called “the problem of quantum mechanics” was to demolish von Neumann’s celebrated theorem that purported to show that “quantum mechanics would have to be objectively false in order that another description of the elementary process than the statistical one be possible”. He then showed that certain predictions of quantum mechanics cannot be reproduced by any ‘local’ theory in which the results of a measurement on one system are unaffected by operations on a distant system with which it interacted in the past. Although “only to be expected”, in Bell’s phrase, the subsequent verification of these predictions was of fundamental importance.

His masterly expositions of the shaky state of the foundations of quantum mechanics, in which he stressed particularly that in the absence of a definition of what constitutes a ‘measurement’ — or ‘experiment’ as he preferred — the predictions are in principle ambiguous, did much to rock the “complacent” views of most physicists. His own work is the most important contribution to ‘quantum philosophy’ since the birth of quantum mechanics, although his clarifications served only to deepen the fundamental mysteries of the subject.

Total honesty and decency characterized all John’s acts. Although he regarded as “good fun” the contacts with the Dalai Lama, the Maharishi, and assorted mystics that resulted from the notoriety of his work on quantum mechanics, he was a private person of great modesty. His many friends will sorely miss his quiet, red-headed and bearded figure padding along the corridors of CERN in sandals, his gentle humour, his delightful use of the English language delivered in soft Ulster tones, his advice, and of course his views of Physics.

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Scissors and helical forks

Robert T. Sauer

THE recognition of DNA by gene-regulatory proteins is often mediated by simple structural motifs that are part of a single protein domain. One such domain is found in the highly conserved ‘leucine-zipper’ family of eukaryotic transcription factors, which includes C/EBP, Fos, Jun and GCN4. The DNA-binding domains of these proteins contain adjacent basic and leucine-zipper regions, each of about 30–40 amino acids. The leucine-zipper region mediates dimer formation via a coiled-coil arrangement of parallel α helices, in which leucines occur every seven residues^{1,2}. The preceding sequence, which is rich in basic residues, has been proposed to make direct contact with DNA, as binding requires both the basic region and the leucine-zipper region, and mutations in either sequence can prevent binding. Recent studies^{3–7}, including those reported by Weiss *et al.*⁶ and Patel *et al.*⁷ on pages 575 and 572 of this issue, now provide evidence which suggests that the basic regions of leucine-zipper proteins assume a stable α -helical structure only when bound to a specific DNA site.

Earlier this year, Talanian *et al.*³ showed that the basic region contains all of the information necessary for DNA recognition. Using a synthetic peptide containing the basic region of GCN4 followed by a Gly-Gly-Cys linker, they observed strong, sequence-specific DNA binding, as long as the peptide dimer was linked by a disulphide bond and the binding experiments were performed at low temperature. Under these conditions, the normal dimerization function of the leucine-zipper region can be replaced by a disulphide crosslink.

The basic region of GCN4, as an isolated peptide or attached to the leucine-zipper region, is about 25 per cent α -helical in solution, as judged by circular dichroism^{3,5,6}. This indicates either that this region contains a short region of stable helix or, more likely, that it consists of an ensemble of partially folded forms⁶. On binding to a specific DNA site, however, the basic region becomes almost completely α -helical^{3,5,6}. So binding of GCN4 peptides to DNA must be accompanied by a significant change in the conformation of the basic region. The conformations of the basic regions of C/EBP, Jun and Jun–Fos also seem to be altered on binding to DNA^{4,7}, presumably as a consequence of a similar coil-to-helix transition. One might worry that the conformational change accompanying DNA binding in all of these systems is an artefact, occurring in the peptide fragments used for these studies but not in the intact leucine-zipper proteins from which they

derive. But peptide fragments from proteins such as GCN4 seem to have specific DNA-binding affinities comparable to those of the intact proteins⁶; this would not be expected if the DNA-binding surface were properly structured in one molecule but not the other. Also, intact GCN4 is reported to show an increase in its helix content, similar to that of the peptides, when it binds to specific DNA⁵.

The α -helical nature of the DNA-bound basic region had been independently predicted by two groups^{5,8}, providing impetus for some of the circular dichroism experiments described above. These predictions were based on the absence of helix-breaking residues such as proline and glycine in the basic region, the periodic distribution of conserved residues in this region, and the invariant spacing between the basic and zipper regions. Moreover, both groups proposed that the DNA-bound protein would have a Y-shaped conformation, with the dimeric leucine zipper forming the base of the Y and the helical basic regions diverging to form the arms.

In the ‘scissors grip’ model of Vinson *et al.*⁸, the two basic regions approach the DNA at a common point near the cleft of the Y, and then track in opposite directions along the major grooves of the dyad symmetric site. Interactions with the major groove are required to account for the known positions of protein–DNA contact^{8–10}. A kink or sharp bend in each α helix allows these regions to follow the curve of the major groove, thereby wrapping around the DNA. The ‘induced helical fork’ model of O’Neil *et al.*⁵ is fundamentally similar to the scissors grip model, although the α helices in this model are not kinked and a specific set of DNA contact residues is proposed. Although the details of these models need to be tested, their basic features are likely to be correct. It therefore seems that the leucine-zipper proteins use a new DNA-binding motif. The scissors grip and helical fork models are also attractive because they provide a rationale for the observed change in conformation that accompanies DNA binding. In solution, the helical arms of the Y would not be stabilized by tertiary interactions, and hence the corresponding basic regions would be expected to be relatively unstructured. In the complex, stabilizing tertiary contacts would be provided by the interactions of the α helices with the DNA.

The conformational transition of the basic region may be an important or even essential feature of the activities of the leucine-zipper proteins. From a thermodynamic point of view, the disorder–order

transition that accompanies DNA binding could provide a mechanism for limiting the free energy of binding, while still allowing maximal specificity of binding. Although the binding energy could also be reduced by interfering with contacts in the complex, this would lead to a reduction in specificity. The basic region might have to be loosely structured in solution for kinetic reasons. In the scissors grip model, the protein forms a type of molecular clamp by encircling the DNA⁸, and large kinetic barriers in the assembly and disassembly of the complex would be expected unless the basic regions were flexible^{4,6}. Weiss *et al.*⁶ point out that a similar situation occurs in the λ repressor, where the N-terminal arms of the protein, which wrap around the DNA in the complex¹¹, are flexible in solution.

Another potential role for the disorder-order transition is suggested by O'Neil *et al.*⁵. They found that the basic region has an intermediate level of helix when bound to nonspecific DNA and speculate that this may allow the protein

to perform an efficient 'search' of the DNA for the specific recognition site. Finally, Patel *et al.*⁷ reason that the conformation of the activation domains of Fos and Jun, which are close to the basic region, may also be altered in the absence of DNA. This, they argue, might provide a way in which regulatory molecules that are not bound to DNA could avoid 'squenching' or competing for components that are needed by the transcriptional machinery.

Conformational transitions are not limited to the basic region of the leucine-zipper proteins. Peptides containing the basic and leucine-zipper regions of GCN4 undergo a concentration-dependent transition in the micromolar range, reflecting a concerted dissociation and unfolding of the coiled-coil structure of the leucine-zipper region⁶. So the peptide is predominantly monomeric and unfolded at the nanomolar concentrations at which specific DNA binding occurs *in vitro*. This should result in a cooperative binding reaction, with DNA binding showing a

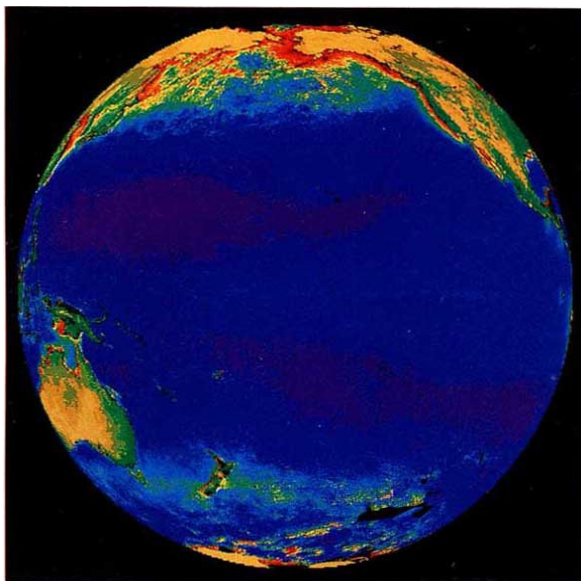
second-order dependence on the concentration of free protein. Does cooperativity of this type operate in the cell? The answer depends on whether the intact protein is predominantly monomeric or dimeric *in vivo*. Because the intracellular concentrations of most leucine-zipper proteins are low, most free proteins would be expected to be monomeric if the behaviour of the peptide is a good model for the intact protein, although binding to non-specific DNA could stabilize the dimer. If the DNA-binding domains of leucine-zipper proteins are actually unfolded in the cell, then one may also wonder whether these regions are complexed with molecular chaperonins, and, if so, whether such interactions have any regulatory role.

Understanding the stability of the paired α helices of the leucine-zipper region is crucial for determining the rules that govern the formation of particular homodimers such as Jun-Jun or heterodimers such as Jun-Fos. But it is still not clear which sequence elements provide the specificity for these interactions, and, indeed, what determines whether these coiled-coil interactions will be parallel or antiparallel. Until recently, most structural information on parallel coiled-coils came from model building or low-resolution diffraction studies on proteins such as tropomyosin. However, higher resolution detail should soon be available from the recently completed crystal structure of the parallel coiled-coil region of GCN4 (E.K. O'Shea *et al.*, personal communication). A high-resolution view of an antiparallel α -helical coiled-coil, roughly 60 Å in length, can be found in the crystal structure of seryl-tRNA synthetase, which was reported last month¹². This structure is stabilized by a 'ladder' of Leu-Leu and Leu-Ile interactions and a variety of inter-helical and intra-helical salt-bridges and hydrogen bonds. Comparison of the structures of these parallel and antiparallel α helices should be helpful in untangling the mysteries of coiled-coils. □

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Solar power to the plankton

IT IS tempting to think of the Pacific Ocean as a vast watery waste that separates Asia and Australasia from the Americas, with little direct effect on the bordering continents. Yet the two-yearly climate cycles associated with the El Niño events bear powerful witness to the importance of the ocean. El Niño events, which can bring drought to Australasia, and excessive rainfall to Peru, are closely tied to cyclical variations in the surface temperature of the Pacific Ocean. Attempts to understand the mechanism relating the two cycles have been



hindered by a disparity between the estimated flux of heat across the ocean-atmosphere interface and that calculated in computer models. Marlon R. Lewis and colleagues, who describe their work on page 543 of this issue, have used images from the NIMBUS-7 satellite to estimate the transparency of the sea surface to solar radiation. The Coastal Zone Color Scanner, an experimental instrument included in the satellite only for testing, proved so successful that its planned one-year life was extended to seven. The image above is a compilation of the full seven-year's data: Lewis and colleagues used just the first year's, which are the most reliable. The image is coded purple, for the most transparent water, through to green and yellow, for the most turbid regions. Penetration of solar radiation through the ocean's most transparent regions means that less of its heat is deposited near the ocean-atmosphere interface than had been supposed. This accounts neatly for the discrepancy between model and estimated sea surface temperatures. Phytoplankton, the main cause of the ocean surface's opacity, are also the principal source, through changes in their abundance, of interannual variations in the atmospheric concentration of carbon dioxide (see U. Siegenthaler's News and Views article, *Nature* 345, 295; 1990). The new work by Lewis and colleagues further underlines the importance of these apparently benign lifeforms in climatic feedback cycles.

R.P.

1. Landschultz, W.H., Johnstone, P.F. & McKnight, S.L. *Science* **240**, 1759-1764 (1988).
2. O'Shea, E.K., Rutkowski, R. & Klim, P.S. *Science* **243**, 538-542 (1989).
3. Talanian, R.V., McKnight, C.J. & Klim, P.S. *Science* **249**, 769-771 (1990).
4. Shuman, J.D., Vinson, C.R. & McKnight, S.L. *Science* **249**, 771-774 (1990).
5. O'Neil, K.T., Hoess, R.H. & DeGrado, W.F. *Science* **249**, 774-778 (1990).
6. Weiss, M.A. *et al.* *Nature* **347**, 575-578 (1990).
7. Patel, L., Abate, C. & Curran, T. *Nature* **347**, 572-575 (1990).
8. Vinson, C.R., Sigler, P.B. & McKnight, S.L. *Science* **246**, 911-916 (1989).
9. Nakabeppu, Y. & Nathans, D. *EMBO J.* **12**, 3833-3841 (1989).
10. Oakly, M.G. & Dervan, P.B. *Science* **248**, 847-850 (1990).
11. Jordan, S. & Pabo, C.O. *Science* **242**, 895-899 (1988).
12. Cusack, S., Berthet-Colominas, C., Hartlein, M., Nassar, N. & Leberman, R. *Nature* **347**, 249-255 (1990).

Space-based gravity tests

Clifford M. Will

WHAT does the future hold, if anything, for experimental studies of gravitation? This was the central question at a recent conference* held to honour the memory of William M. Fairbank, one of the most enthusiastic and forceful advocates for experimental gravitation, who died last year¹. Noted for several discoveries in low-temperature physics, Fairbank turned his attention during the 1960s to the application of cryogenic techniques to precision gravity experiments, and his

masses of the Earth and Moon) were verified to accuracies ranging from 1 to 0.01 per cent. And observations of the decaying orbit of the remarkable binary pulsar confirmed the theory's predictions for gravitational-wave damping^{2,3}.

For all the painstaking and time-consuming work that went into the experimental tests of the golden era, they were somehow easy, for they were done at a time when advances in technology (atomic clocks, radio and radar astronomy, space

The frame-dragging effect responsible for this precession is a rotation of the axes of local inertial frames relative to distant stars caused by the rotation of the Earth (see figure). The effect is sometimes called gravito-magnetism because it is analogous to the precession of a magnetic moment outside a rotating charged body. Apart from being an important testable prediction of general relativity, it plays a central role in supermassive-black-hole models for the formation of jets in quasars, and the notion that distant matter can induce rotation of local inertial frames has obvious machian overtones. Thus the direct detection of this effect is a goal worth reaching, despite the difficulties. The required precision implies measuring a precession rate which is another near zero: 10^{-17} radians per second.

In the 1960s and 1970s, what was needed to achieve this seemed beyond the pale: the 4-cm-diameter gyroscopes would have to be homogeneous and spherical to a few parts in 10^7 ; the spacecraft carrying the experiment would have to be free of drag (residual accelerations less than 10^{-12} g); an onboard telescope would be needed, capable of providing a reference direction stable to 0.1 milliarcseconds per year; and so on. But recently the GP-B laboratory successfully conducted an Earth-bound test of the integrated gyroscope-cryogenics-telescope system (M. Tater, Stanford University), showing that many of the requirements have now been met and that others will follow once the device reaches the low-gravity environment of space. The current plan calls for an orbiting mission around 1996.

But GP-B is not the only space-based gravitation experiment being planned. Others for the next decade are at various stages of development: some are just conceptual; for others, the hardware is being developed. Their goals include detection of low-frequency gravitational waves (seismic noise restricts Earth-bound detectors to seeking frequencies above 1 hertz), orders-of-magnitude improvement of current tests of general relativity, and probes of higher-order relativistic gravitational effects. Some of the projects

Experiment	Quantity measured	Accuracy proposed
Satellite test of equivalence principle	Differential acceleration of different materials	10^{-17}
Optical interferometer in space	Light deflection	10^{-6}
Laser-ranged Earth satellite	Frame-dragging of orbit plane	2×10^{-3} arcsec yr ⁻¹
Atomic clock on Solar Probe	Gravitational redshift	10^{-10}
Microwave interferometer in space	Gravity-wave strain at 10^{-2} Hz	10^{-18}
Laser interferometer in space	Gravity-wave strain at 10^{-4} Hz	10^{-21}
Gravity gradiometer in space	Curvature tensor	10^{-19} g cm ⁻¹

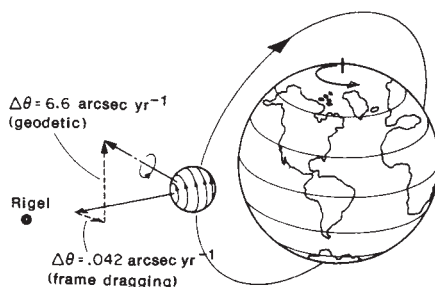
Stanford laboratory soon became a centre for such work. This included the construction of bar detectors for gravitational waves, attempts to measure the free fall of positrons, and an experiment to measure the einsteinian 'dragging of inertial frames' by the rotating Earth using an orbiting gyroscope.

Coincidentally, the conference also came within two months of the 75th anniversary of Einstein's final formulation of the general theory of relativity. Based on the principle of equivalence, or the equality of free-fall acceleration of different bodies, general relativity accounts for gravity as a consequence of curved space-time. Although the theory was pretty much in hibernation for its first 45 years, the past 30 have witnessed a renaissance of the subject, not least in the area of experimental gravitation. In fact 1960–80 was something of a golden era for testing relativity, during which most of the theory's predictions for effects in the Solar System (light deflection, gravitational redshift, retardation of light, the advance of Mercury's perihelion and the equivalence principle for the inertial and gravitational

exploration) met the demands for checking the validity of general relativity, which arose from astronomical discoveries. The necessary experiments *could* be done with good precision, and so they were.

In contrast, the experiments envisaged for the future require almost heroic advances in technology and precision techniques to reach the required sensitivities. *Near Zero*, a *Festschrift* for Fairbank⁴ published in 1988, neatly characterizes the requirements for many of these experiments: near-zero temperature (10^{-3} K noise temperature in gravity-wave antennae); near-zero magnetic field (10^{-7} gauss in the gyroscope experiment); and near-zero acceleration (10^{-11} g for a satellite test of the equivalence principle). Why? Because the effects to be measured are also near zero.

Nothing illustrates this better than the gyroscope experiment, denoted Gravity Probe B (GP-B) by NASA, which was described in detail at the meeting (C.W.F. Everitt, Stanford University). The plan is to place a set of four superconducting-niobium-coated, quartz gyroscopes into polar Earth orbit to measure the predicted 42 milliarcsecond per year precession of the spin axes to better than 0.5 per cent.



Precession effects due to relativity in a gyroscope experiment orbiting the Earth. Detecting the 42 milliarcsecond change each year due to 'frame dragging' will require remarkable, but achievable precision. (From C.W.F. Everitt in ref. 4, page 589.)

* *Relativistic Gravitational Experiments in Space*, Rome, 10–14 September 1990.

discussed at the meeting are outlined in the table.

If these experiments are so difficult and take so long, and require access to space (which has lately become such a precious commodity), why bother to do them? The answer, in the case of gravity-wave detectors, is that a new astronomical window will open up, revealing information about violent and dynamic cosmic events. Furthermore, only space-based detectors can 'see' the known sources of gravity waves, such as close binary systems.

As for the basic notion of gravitational tests, general relativity is a fundamental theory of nature, and should be given the best empirical backing we can provide. More importantly, its predictions are fixed; the theory contains nothing that can be adjusted to accommodate any experi-

mental deviations. Thus any test of general relativity is potentially a deadly test, for any verified deviations would kill the theory. As his quest for fractionally charged free quarks showed, Fairbank loved to try experiments that might overthrow conventional wisdom; one suspects that even an outside chance of demolishing general relativity would have kept him in the game for many years to come. □

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1. Cabrera, B. *Nature* **342**, 125 (1989).
2. Will, C.M. *Phys. Rep.* **113**, 345 (1984).
3. Will, C.M. *Was Einstein Right?* (Basic Books, New York, 1986).
4. Fairbank, J.D. et al. (eds) *Near Zero: New Frontiers of Physics* (W.H. Freeman, New York, 1988).

PLANT PATHOLOGY

The race for resistance genes

Geoffrey North

PLANTS have defence mechanisms by which they can resist infection by potentially pathogenic microorganisms. Like the immune systems of higher animals, these mechanisms work by first recognizing foreign molecules produced by the invading pathogen. The recognition repertoire of plants, however, lacks the developmental flexibility of animal immune systems, and is set for the life of a particular plant by the collection of so-called 'resistance' genes it happens to inherit. The cloning of resistance genes is an important goal in plant pathology, and on the evidence of a recent meeting* it should not be long before it is achieved.

Resistance genes commonly exhibit what are known as 'gene-for-gene' interactions with specific genes carried by the pathogen, which are perhaps misleadingly referred to as 'avirulence' genes. Gene-for-gene interactions are recognized by a simple pattern of inheritance such that plants carrying a particular resistance gene can recognize, and are thus resistant to, pathogens carrying the complementary avirulence gene, but if either resistance gene or avirulence gene is absent a virulent infection ensues. The resistance gene is assumed to code for some kind of receptor molecule which signals the presence of the product of its complementary avirulence gene, setting off a 'hypersensitive reaction' by which the plant can isolate the pathogen at the site of infection and prevent its spread throughout the plant.

Avirulence genes are thus named because their presence prevents virulent infections on host plants carrying the

appropriate resistance genes. It is presumed that pathogens do not carry avirulence genes to alert host plants to their presence, and work on the pathogen *Xanthomonas campestris*, which causes bacterial spot disease of tomato and *Capsicum*, provides evidence that at least some avirulence genes are required for full virulence of the pathogen on susceptible hosts (Kearny, B. & Staskawicz, B. J. *Nature* **346**, 385–386; 1990).

This property may explain the high degree of conservation of the avirulence gene *avrBs2* among *X. campestris* strains, and why its complementary resistance gene *Bs2* provides stable field resistance to bacterial spot disease in *Capsicum* (B. J. Staskawicz, University of California, Berkeley). The hope is that the *Bs2* gene will work similarly in other crop plants, to which it could be transferred when it has been cloned.

A number of routes are being taken to clone plant resistance genes in the absence of a defined biochemical product. For example, efforts are being made to exploit the advantages of the small crucifer *Arabidopsis thaliana*, currently the favourite subject of the plant molecular geneticist (F. Ausubel, Massachusetts General Hospital, Boston). Certain *Arabidopsis* lines have resistance genes corresponding to avirulence genes carried by particular strains of *Pseudomonas syringae*, and as a first step Ausubel and colleagues are attempting to isolate mutants that have lost resistance. In particular, they would like to obtain plants with deletion mutations in a resistance gene, which would enable them to apply the technique of 'subtractive hybridization'. Here, genomic DNA with the deletion mutation is hybridized to DNA from the wild-type

parental plants in the hope that the non-hybridizing DNA, which can be cloned, would be enriched in sequences representing the deletion. The technique has already been used to clone an *Arabidopsis* gene for an enzyme of gibberellin biosynthesis; the problem, however, is the lack of a straightforward general way of inducing deletion mutations. Using a screen with the chemical mutagen ethylmethanesulphonate, an *Arabidopsis* mutant has been obtained that has lost resistance to a particular strain of *X. campestris* (M. J. Daniels, John Innes Institute, Norwich). But cloning the site of mutation is not an easy step.

A subtractive hybridization procedure is also being used to clone tomato resistance genes to the fungal pathogen *Cladosporium fulvum* (M. Dickinson, John Innes Institute). In this case the subtraction is done not with genomic DNA but with complementary DNA copied from the messenger RNA of plants with and without a particular resistance gene. A number of cDNAs have been identified that seem to be specifically expressed in tomato plants carrying resistance gene *Cf2*, though as yet none has been shown to be the product of the *Cf2* gene.

Other groups are using an approach well known to human geneticists — that of mapping resistance genes relative to polymorphic markers, followed by 'chromosome walking' to the resistance gene. One requirement is a dense enough map of polymorphic markers throughout the genome, and a restriction fragment length polymorphism (RFLP) map is being built up for lettuce and used to map the position of resistance genes to the downy mildew fungus *Bremia lactucae* (R. Michelmore, University of California, Davis). A similar approach is being used to map and ultimately clone tomato resistance genes to *Pseudomonas syringae* pv *tomato* (S. Tanksley, Cornell University). Both of the groups concerned reported how the construction of a map of polymorphic markers is being facilitated by the use of a new technique based on the polymerase chain reaction (PCR). This simply involves the cloning by PCR of regions of the genome picked out by random primers: several regions are picked out by any one set of primers, thus increasing the chance of finding a polymorphism relative to a search using a single-copy probe.

The practical benefits of cloning resistance genes are likely to be considerable. Moreover the enterprise will probably provide insight into the poorly understood processes of signal reception and transduction in plants, as well as into the evolution of resistance in the arms-race between plants and their pathogens. □

Geoffrey North is Deputy Biology Editor of *Nature*.

* The 5th International Symposium on the Molecular Genetics of Plant-Microbe Interactions, Interlaken, Switzerland, 9–14 September 1990.

Making light of polymers

Lewis Rothberg

THE new light-emitting diode (LED) described by Burroughes *et al.* on page 539 of this issue¹ is one of several applications now being found for semiconducting polymers. Such an LED could be used more conveniently than conventional silicon-based ones for making large-area flat panel displays. And the processing method means that the polymer can easily be spun onto its substrate circuitry directly from solution. The low temperature used in processing also means that there is no risk of damage to other components in the circuit.

Many conjugated polymer molecules behave as quasi one-dimensional semiconductors, owing to the delocalization of π -orbital electrons along the organic backbone chain. The dimensionality, composition and morphology of these materials cause them to behave in very different ways from conventional silicon semiconductors. The hope is that the mechanisms responsible for these differences could allow the organic polymers to be used in novel electrical and optical devices. Besides the LED prepared from poly(*p*-phenylene vinylene) (PPV) by Burroughes *et al.*¹, there have been reports of polymers doped to be as conducting as metals² and of polyacetylene induced to have giant nonlinear optical susceptibilities³, necessary for the development of active optical components.

At a more fundamental level, there remains a great deal of mystery about the nature of the charge carriers in these organic semiconductors. The softness of the polymeric lattice leads to strong coupling between the polymer's electrons and vibrations (phonons) such that it is energetically favourable for the local molecular structure to deform around free charges to form lattice-stabilized quasiparticles^{4,5}. The spins and effective masses of these quasiparticles can be very different from those of free electrons and holes, and it is the quasiparticles that are thought to be responsible for the conductivity (and photoconductivity) of the conjugated polymers.

In the case of PPV, Burroughes's colleagues⁶ have previously shown that a neutral quasiparticle, the polaronic exciton, is responsible for the material's photoluminescence, which is spectrally identical to the recombination luminescence

observed in the LED. As this photoluminescence presumably comes from intrachain electron-hole pairs formed by the absorption of a photon, the authors argue that the injected electrons and holes in the LED travel as 'polarons' which, when they encounter an oppositely charged polaron on the same chain,

GEOLOGY

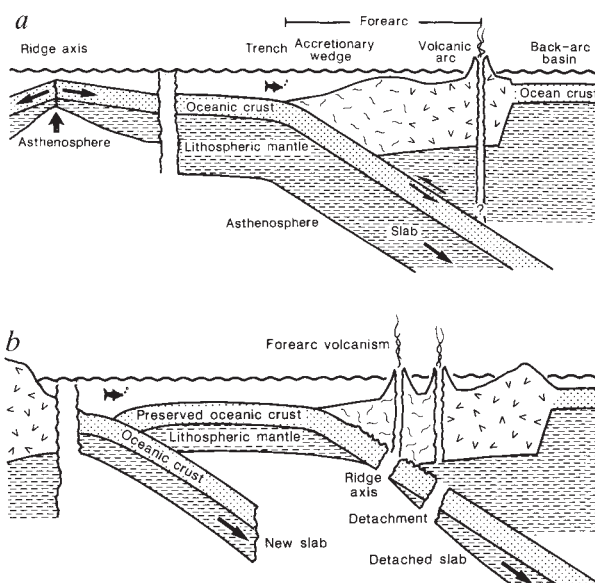
A reprieve for ocean crust

Norman H. Sleep

A KEY aspect of plate tectonics is that oceanic crust is formed at mid-oceanic ridge axes, moves horizontally across ocean basins, and is then subducted back into the mantle at trenches. Yet some oceanic crust avoids this fate and is exposed as ophiolites within island arcs or

transiently form quasi one-dimensional excitons which decay radiatively. This idea contrasts with the results from the previous optical work⁶ which suggest that bipolarons are the most stable quasiparticles in PPV. The resolution to this intriguing difference will no doubt lead to a better understanding of quasiparticle transport in conjugated systems. □

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Birth of an ophiolite: *a*, normally the ridge axis is far from the trench. The oceanic crust is carried down with the slab. The sinking of the cold dense slab provides a driving force for the plate motion. Note that the oceanic crust in the backarc may be preserved by virtue of being attached to the crust of the island arc. *b*, The subduction zone on the right dies when the ridge is subducted. A new subduction zone begins on the left. The preserved oceanic crust will be uplifted when the continent to the left collides with the subduction zone.

on continents. Ophiolites were described in many localities before their significance was understood. The connection with oceanic crust was made in the late 1960s: rocks dredged from the sea floor were found to be similar to those in ophiolites; the layering of oceanic crust as detected by seismic studies is similar to the observed structure of ophiolites (from marine sediments through crustal rocks of basalt

composition down to the mantle); and ophiolites clearly formed in zones of extension similar to those imagined to occur at ridge axes. The processes by which oceanic crust becomes ophiolites, however, have remained poorly understood. In new work, Jilles van der Beukel (*Tectonics* 9, 825–844; 1990) investigates a mechanism of ophiolite emplacement where a ridge axis is unusually close to a trench and young oceanic crust fails to subduct.

As ophiolites on land provide better exposures than could be obtained from any conceivable marine drilling programme, it is essential to know how far geological studies of ophiolites can be extrapolated to oceanic crust or, conversely, whether ophiolite preservation mechanisms create a biased sample. Clearly, ophiolites are unusual in the sense that they escaped subduction. Thus, it is often argued that the crust is also intrinsically different and did not form at a normal mid-oceanic ridge. For example, 'autochthonous' ophiolites are formed by spreading in the forearc and backarc basins found on the overriding plate at subduction zones, and along new passive margins, and survive by being attached to buoyant continental crust.

In contrast, there are two-step mechanisms whereby normal oceanic crust is emplaced in the forearc and then uplifted and often exposed as the overriding plate in an arc-continent collision. For example, plate reorganizations that involve formation of a new subduction zone within oceanic crust, either within a plate or by the change of an oceanic trans-

1. Burroughes, J. H. *et al.* *Nature* **347**, 539–541 (1990).
2. Naarman, H. & Theophilou, N. *Synth. Metals* **22**, 1 (1987).
3. Fann, W.-S. *et al.* *Phys. Rev. Lett.* **62**, 1492–1495 (1989).
4. Su, W.-P., Schrieffer, J. R. & Heeger, A. J. *Phys. Rev. Lett.* **42**, 1698 (1979).
5. Feldman, A. *et al.* *Phys. Rev.* **B26**, 815 (1982).
6. Friend, R. H., Bradley, D. D. C. & Townsend, P. D. *J. Phys.* **D20**, 1367 (1987).

form fault from a strike-slip to a convergent boundary, will by their kinematics leave oceanic crust in the forearc.

van der Beukel's mechanism — failed subduction of young oceanic crust (formed at mid-ocean ridges) — is such a two-step mechanism. However, it is not a geometrical requirement of the gross motions of plates. In part, the mechanism is an inference from geometrical plate tectonics (see figure): mid-ocean ridges spread more or less symmetrically, whereas subduction is one sided. Thus, ridge axes gradually approach trenches so that progressively younger crust enters the trench. If the component of the full spreading rate in the direction of subduction is less than half the subduction rate, the ridge axis collides with the trench and somewhat slower subduction of the plate on the other side of the ridge axis begins. van der Beukel envisages that around the time of the collision, the subducting plate fails mechanically and the slab becomes detached. Subduction restarts on the other side of the ridge axis, preserving a block of the crust in the forearc. The preserved oceanic crust may be normal or it may show chemical effects of its encounter with the arc.

The position of slab detachment and the position where subduction restarts are consequences of the thermal structure and stresses within the subducting plate. (It is geometrically possible for the ridge axis to be peaceably subducted so that no ophiolite is created.) van der Beukel calculates the thermal structure of the subducting plate and the overlying arc and then evaluates what causes the failure of the plate with a flexural model. The main prediction is the position where detachment occurs at depth and thus the geometry of the preserved piece of oceanic crust. For a variety of spreading rates and rheological laws of the plate and the fault zone, detachment occurs at depths between 50 and 100 km. The outboard tip of the arc, the toe of the accretionary wedge, may or may not override the ridge axis.

The models have one frustrating aspect: the key prediction, the depth at which detachment occurs beneath the arc, is not easily determined by either geophysical remote sensing or the geology of exposed sections. van der Beukel's extension of the models to three dimensions is more immediately relevant. Plate failure and ophiolite preservation occur when the colliding ridge is a small part of a much larger plate driven by subduction of older lithosphere elsewhere. This explains the occurrence of young ophiolites in southern Chile. It remains to be seen whether ridge-trench collisions have a large or small role in preserving ophiolites globally. □

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Phospholipid transfer market

James E. Rothman

PHOSPHOLIPID-transfer proteins (originally termed 'exchange proteins') have the remarkable capacity to exchange lipid molecules between membranes without altering their own total lipid content. In the two decades or so since they were discovered¹, these enigmatic molecules have posed a puzzle which, with the remarkable report by Bankaitis *et al.* on page 561 of this issue², is now partly resolved.

The puzzle is this: why should all eukaryotic cells have in their cytoplasm a battery of different transfer proteins that (at least *in vitro*) are incapable of directing net lipid movement between organelles? It is all the more perplexing because transfer proteins will use virtually any biological membrane or synthetic lipid bilayer as substrate. Could such non-specific proteins possibly mediate the kinds of vectorial movements of lipids between specific organelles that would be useful in membrane biogenesis and recycling? Apparently, yes, as Bankaitis *et al.* demonstrate. With the surprising identification of one of the phospholipid-transfer proteins of yeast as the product of a secretion gene, *SEC14*, a specific biological function for them has at last been found, most probably the vectorial removal from, or delivery of lipids to, the Golgi apparatus in order to stimulate protein secretion.

Three kinds of phospholipid-transfer protein, differing in their headgroup specificities, have been purified from mammalian cytosol and their properties investigated³. The phosphatidylcholine (PtdCh) transfer protein will catalyse the exchange between bilayers of only PtdCh. The phosphatidylcholine/phosphatidylinositol (PtdCh/PtdIns) protein exchanges both PtdCh and PtdIns. And a nonspecific transfer protein will exchange all species of phospholipids (and also sterols).

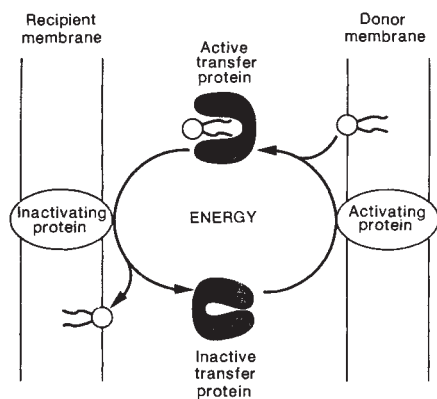
All three kinds of transfer protein seem to work by acting as diffusible carriers of one lipid molecule at a time, according to a simple mechanism: when a lipid-laden transfer protein encounters a membrane surface, it discharges the lipid into the outer leaflet of the bilayer, and does not dissociate until its lipid-binding site is once again full. No net transfer of lipid between membranes is possible with this mechanism, and only the leaflets of membranes facing the cytoplasm are affected. Also, no energy is needed for this exchange process. In the case of the PtdCh/PtdIns transfer protein, the unusual dual headgroup specificity for two dissimilar phospholipids results from a single lipid-binding site that may be occupied either

by PtdCh or by PtdIns, but not by both at the same time. This enables the protein to achieve a net transfer of PtdCh or PtdIns between membranes without affecting total lipid content when two membranes initially differing in their PtdCh:PtdIns ratio are mixed.

To test whether transfer proteins have any essential function in cell biology, Dowhan and colleagues⁴ undertook to purify the PtdIns/PtdCh transfer protein from baker's yeast. They obtained a cytosol-derived protein of relative molecular mass 35,000 which accounted for most or all of the PtdIns and PtdCh transfer activity of crude yeast cytosol. Using probes derived from a partial amino-acid sequence, they obtained a DNA clone of the corresponding structural gene (termed *PIT1*). Disruption of the *PIT1* gene by homologous recombination was lethal, implying that the PtdCh/PtdIns-transfer protein performs an essential task in cell biology. But what could this be?

The answer has come from the confluence of this work with an independent line of investigation by Bankaitis and colleagues⁵, who had cloned and sequenced the *SEC14* gene, also from yeast. The *sec14-1ⁿ* mutant is temperature-sensitive⁶, and has an unusual phenotype: at the restrictive temperature, secretion from the Golgi to the cell surface ceases, but transport from the endoplasmic reticulum (ER) into the Golgi continues. The secretion block is accompanied by a massive accumulation of Golgi membranes. Normally, the existence of a Golgi in yeast has to be taken almost as an article of faith, the membranes being sparse and elusive. But *sec14-1ⁿ* cells accumulate Golgi membranes as their principal cytoplasmic organelle.

In their report², Bankaitis, Dowhan and coworkers note that the published⁷ N-terminal protein sequence of the PtdCh/PtdIns-transfer protein is identical to that of protein product of the *SEC14* gene predicted from the published⁸ sequence. They go on to show that the *PIT1* clone, in single copy, will restore growth to *sec14* mutant, and that the nucleotide sequences of the *SEC14* and PtdCh/PtdIns-transfer protein clones are identical. Consistent with the hypothesis that the lipid transfer activity of the protein SEC14p is the primary defect in the temperature-sensitive *sec14* mutant, the PtdIns transfer activity of crude cytosol prepared from *sec14-1ⁿ* cells was entirely temperature sensitive *in vitro*. As expected, the PtdCh-transfer activity of yeast cytosol was also thermolabile, but interestingly the PtdCh-transfer activity of sec14p is more thermosensitive



Possible energy-driven cycle resulting in net lipid flow from a lipid donor membrane housing an activating protein (enzyme) to a recipient membrane containing an inactivating protein (enzyme). Inactivating and activating proteins are purely hypothetical.

than its PtdIns-transfer activity.

Bankaitis *et al.*² propose that the PtdCh/PtdIns-transfer protein is needed either to remove phospholipids from the Golgi, or alternatively to deliver them to the Golgi, in order to maintain the secretory pathway in an active state. Concerning delivery, they suggest that the transfer of PtdIns to the Golgi from its site of synthesis in the ER by the protein (presumably in exchange for Golgi PtdCh) may be needed for Golgi function. But this seems an *ad hoc* explanation, as it is hard to understand why the existing vesicle flow from the ER to the Golgi would not suffice to provide the Golgi with PtdIns. Nor is there any particular reason to envisage a special role for PtdIns in Golgi transport, as distinct from, say, exit from the ER, because in the *sec14* mutant the first not the second process is disrupted.

Thus, at first glance, a role for the transfer protein in removing phospholipid from the Golgi would seem the more attractive. The Golgi constantly receives a massive input of lipid in the form of transport vesicles derived from the ER membrane during secretion. Wieland *et al.*⁷ have estimated that, in animal cells, half of the phospholipid of the ER is transported into the Golgi in these vesicles every 10 minutes; because the total surface area of the ER is 5–10 times that of the Golgi, this means that there must be a highly active mechanism for removing most of the phospholipid from the Golgi every minute or two and returning it back to the ER. Any disruption of this mechanism would lead to a swift increase in the surface area of the Golgi, as is observed in the *sec14* mutant, because of the accumulation of unrecycled phospholipid bilayer. A block in vesicular transport within the Golgi (but not from ER to Golgi) might well be a secondary consequence of this accumulation, as the activities of many membrane-bound enzymes are affected by lipid content and composition, and undoubtedly a

number of (as yet unknown) membrane proteins are required to act in concert to achieve vesicular transport.

Wieland *et al.*⁷ also note that unidirectional protein transport from the ER to the Golgi using an unselective, bulk-flow mechanism might require that much of the necessary recycling of phospholipids proceeds by a non-vesicular mechanism, and they raise the possibility that phospholipid-transfer proteins may provide this route. The new findings of Bankaitis *et al.*² are in striking, if only superficial, agreement with this prediction, which if correct implies that other transfer proteins must carry phospholipids other than PtdCh and PtdIns back to the ER. There would then also have to be some sort of flip-flop mechanism in the Golgi membrane to provide the luminal leaflet with access to the transfer proteins for recycling.

Whatever the role in secretion of phospholipid transfer by the PtdCh/PtdIns-transfer protein proves to be, the results of Bankaitis *et al.*² point to a biological specificity for the Golgi that has no basis in the behaviour of the pure transfer protein. This strongly implies that there are other portions of a lipid-transfer machinery that have been missed, and that the diffusible transfer proteins operate together with compartmentalized membrane proteins to provide the directionality and specificity needed for an organized traffic pattern in which there is a net non-vesicular flow of lipids between sites such as the ER and Golgi.

For example (see figure), in the case of the recycling of phospholipid from Golgi to ER, the ER might contain a membrane protein that binds the soluble transfer protein and causes it to discharge its bound lipid, while releasing the transfer protein in a reversibly modified (inactive) form in which its lipid-binding site is empty. When the inactive but still diffusible transfer protein later encounters a specific activating protein in the Golgi membrane, it would be reactivated and then load up with Golgi phospholipid for another cycle. Either the inactivation or the activation step (or both) would require the input of energy, the ultimate purpose being to enable the transfer protein to do the key thing it cannot do by itself: dissociate empty from a membrane. □

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1. Wirtz, K. & Zilversmith, D. J. *biol. Chem.* **243**, 3596–3602 (1968).
2. Bankaitis, V.A., Aitken, J.R., Cleves, A.E. & Dowhan, W. *Nature* **347**, 561–562 (1990).
3. Wirtz, K. & Gadella, T., Jr *Experientia* **46**, 592–599 (1990).
4. Aitken, J.E., van Heusden, G.P.H., Temkin, M. & Dowhan, W.J. *J. biol. Chem.* **265**, 4711–4717 (1990).
5. Bankaitis, V.A., Malehorn, D.E., Emr, S.D. & Greene, R. J. *cell Biol.* **108**, 1271–1281 (1989).
6. Novick, P., Field, C. & Schekman, R. *Cell* **21**, 205–215 (1980).
7. Wieland, F. *et al. Cell* **50**, 289–300 (1987).

The magic bullet

THE great era of comic films established the custard pie as an item of ballistics rather than food. Daedalus is now reviving this charming fantasy. He points out that modern custard, essentially a suspension of corn starch in water, can have strongly non-linear viscosity. It yields to small loads, but can lock almost solid under strong or sudden impact forces. It would therefore make an interesting bullet: capable of delivering a heavy blow, but flowing freely after the impact.

Now ballistic impact has recently been used as a way of getting foreign DNA into cells. Tiny gold or tungsten spheres are coated with the DNA and fired into the target tissue; some of the cells survive the assault and accept the DNA. Daedalus reckons that small droplets of DNA-loaded custard would be even better. Hardened momentarily by the impact, they would penetrate the cell, and then lapse into liquid once more. DNA and nutrient starch would be distributed round the cell without further trauma. Such a relatively gentle injection technique might even be scaled up sufficiently for medical use. Vaccines and other medicaments could be fired through the skin of those patients who seriously don't like needles. Problems of syringe-sterility would vanish too.

The high speed of the pharmaceutical custard droplets would even let them be delivered from some distance away. Daedalus had amusing visions of a whole queue of patients standing with their right sleeves rolled up while the doctor sprayed them with ballistic vaccine like a medical St Valentine's day massacre.

He then recalled how hampered modern policing is by the lack of appropriate weapons. Against rioters, terrorists, hijackers and so on the traditional truncheon is ineffective, while military firearms are too lethal and indiscriminate. But a suitable ballistic custard, loaded with anaesthetic and formulated to be fired from a specially adapted rifle, could solve the whole problem. The aerodynamic forces of flight would probably fragment it into a tight cloud of droplets. On hitting the victim, each droplet would momentarily harden, penetrating his clothing and skin. The resulting minor wound, a mere pin-prick in itself, could deliver a powerful pharmaceutical blow. A liquid missile has such a high density that even a small droplet could carry enough anaesthetic to send a determined aggressor rapidly to sleep.

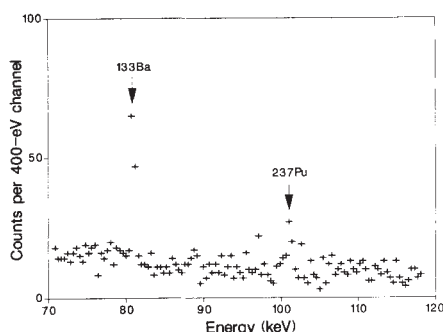
The custard bullet will be warmly welcomed by police marksmen. At last their lonely burden of responsibility will be lifted. They can hit the innocent with the guilty, knocking out all concerned, then collect up the entire unconscious assembly and leave the apportionment of blame and injury to the courts.

David Jones

Leukaemia risk and plutonium

SIR—Müller in Scientific Correspondence¹ suggests that a selective retention of actinides by the male gonads may induce leukaemia in the children of people whose work involves processing these materials. It is commonly assumed for the purposes of radiological protection that most plutonium entering the blood migrates to the liver and skeleton, with only 0.035% being deposited in the testes². This value is based on the observed behaviour of plutonium in various animal species and on concentrations in tissues that were removed at autopsy from three human subjects injected with ²³⁹Pu citrate 5–160 days before death from pre-existing disease³.

The low uptake is further supported by our recent investigations following intravenous injection of ²³⁷Pu as citrate in a



Gamma-ray spectrum recorded with a semiconductor detector viewing the testes, showing a well-defined peak from skeletal ¹³³Ba (81 keV) but only a weak response from ²³⁷Pu (101 keV).

healthy volunteer aged 62 years. This isotope, decaying by electron capture with a half-life of 45.3 days⁴, is a valid metabolic tracer for the long-lived α -emitting isotopes of Pu, despite its much greater specific activity⁵. It was produced by α -irradiation of uranium, which also generated low levels of the α -emitting isotopes⁶. The injection of 1.4 kilobecquerels of ²³⁷Pu led to an acceptable estimated effective dose equivalent of 0.3 millisieverts, of which more than 99% arose from the impurities. The main object of our experiment was to study patterns of excretion, clearance from blood, and hepatic and skeletal uptake of Pu isotopes.

The assessment of gonadal uptake was conducted inside a heavily shielded room, 28 days after the injection, using a high-purity germanium detector 50 mm in diameter and 20 mm thick, whose proximal planar surface was 5 mm behind the beryllium entrance window. The subject supported himself on hands and knees, with thighs separated sufficiently to allow location of the window within 5 mm of the posterior surfaces of the scrotum. Local close-fitting graded shielding (1 mm

cadmium + 2 mm lead) around the curved surfaces of the crystal housing reduced interference from skeletal ²³⁷Pu. Part of the γ -ray spectrum recorded during an initial 20-min measurement is shown in the figure, in which most of the response arises from skeletal ¹³³Ba, injected 2½ years previously in a study of normal bone mineral metabolism; deposition of barium in the testes is negligible (1.7×10^{-5} of body content⁷). The ¹³³Ba is responsible for the prominent peak at 81 keV, superimposed on the continuum of scattered radiation from other lines (mainly 356 keV) in its spectrum. The principal photon emissions from ²³⁷Pu are the K α , X-rays at 101 keV; there is no well-defined feature at this energy in the figure but the response in the range 100–102 keV exceeds that in the adjacent continuum by 0.92 ± 0.26 (s.d.) counts per min per keV. This elevation cannot be ascribed solely to ²³⁷Pu in testes because the response from ¹³³Ba shows that discrimination against skeletal deposits was incomplete.

A second 20-min measurement was therefore made on the same day, with minor postural changes, intended to reduce irradiation of the unshielded window by skeletal activity without impairing detection of ²³⁷Pu in the testes. On this occasion, the elevation was only 0.31 ± 0.21 counts per min per keV.

A crude calibration, with estimated accuracy $\pm 30\%$, was effected with a mock testicle, conforming roughly to the dimensions given for reference man⁷ and containing a solution of ²³⁷Pu standardized relative to the injected quantity; on that basis the smaller elevation corresponded to a gonadal deposit of $0.10 \pm 0.07\%$. Clear evidence of ¹³³Ba (81 keV) persisted in the second spectrum, at about 50% of its intensity in the first, and so this estimate must exaggerate the true deposition in our subject. We conclude that there is nothing to invalidate existing assumptions² concerning testicular uptake of plutonium.

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1. Müller, W.A. *Nature* **345**, 483–484 (1990).
2. International Commission on Radiological Protection, Publ. 30 Part 1 Ann. *ICRP* **2**, No. 3/4 (1979).
3. International Commission on Radiological Protection, Publ. 48 Ann. *ICRP* **16**, No. 2/3 (1986).
4. International Commission on Radiological Protection, Publ. 38 Ann. *ICRP* **11–13**, 1159 (1983).
5. Talbot, R.J. et al. *Health Phys.* **59**, 183–187 (1990).
6. Whittaker, B. *Nucl. Inst. Meth. Phys. Res.* **223**, 531–534 (1984).
7. International Commission on Radiological Protection, Publ. 23 Report of the Task Group on Reference Man (Pergamon, Oxford, 1975).

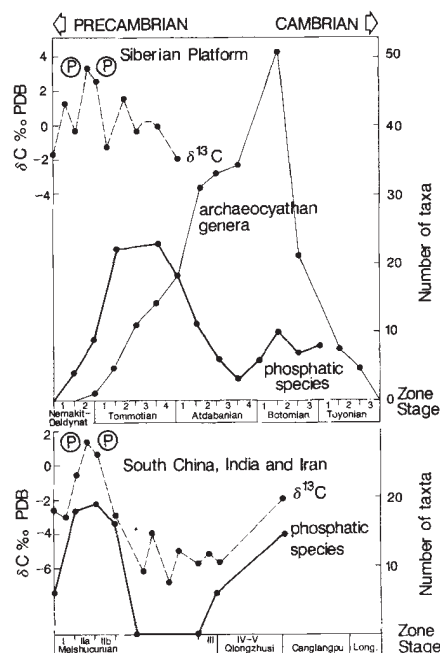
Nutrients in the early Cambrian

SIR—The Precambrian/Cambrian boundary interval is marked by explosive evolution of invertebrate taxa, including the first biomineralized skeletons and by major carbon isotope excursions. A comparison of recent data from each of these lines of evidence in Siberia and south China to Iran (see figure) implies that evolutionary patterns may have been related to shifts in nutrient supply. An early phase of rising productivity and phosphogenesis was accompanied by the initial diversification of phosphatic skeletons. The explosive evolution of oligotrophic sponges with calcitic skeletons, and increasing depths of bioturbation in the sediment, occurred during a later phase of nutrient depletion.

Studies of carbon-isotope stratigraphy across Asia¹ have prompted this question of nutrient supply (especially phosphorus) to the oceans. Changes in $\delta^{13}\text{C}$ can be related to patterns of primary productivity and nutrient supply in modern lakes and oceans. In the Nemakit–Daldynian stage, these isotopes show an interval of increasingly heavier $\delta^{13}\text{C}$ (raised productivity), rapidly replaced by lighter isotopes (lowered productivity) close to the base of Tommotian stage in Siberia, and at a similar horizon from south China to Iran. As major phosphogenic events straddle this turning point (P in the figure), huge fluxes in nutrient supply may have occurred. Sedimentological data suggest this high primary productivity took place in shallow carbonate lagoons, perhaps fed with phosphorus by evaporative reflux from the open ocean. Productivity seems to have declined after the burial of phosphorites across Asia and during each of the great transgressions of the early Cambrian. This decline may have been related to reduced circulation within saline, stratified water masses that flooded the shelves.

Recent ecological observations indicate that biological innovations during the Cambrian explosion may have further depleted nutrient supply. For example, greater depth of burrowing, increased grazing of cyanobacterial and algal mats, the production of larger faecal pellets and larger phytoplankton cells all enhance the removal of nutrients and carbon into sediments². Deep bioturbation was not extensive until about Atdabanian times³.

This model of nutrient glut followed by nutrient depletion is consistent with the contrasting histories of phosphatic skeletons and archaeocyathan sponges through the early Cambrian. The former were phosphophilic, associated with more open-marine, eutrophic conditions. At low latitudes, phosphatic species declined in diversity after the initial phosphogenic stages of the Cambrian explosion⁴, while



Contrasting diversity of oligotrophic archaeocyathan genera and 'phosphophilic' phosphatic skeletal species, compared against published carbon isotopes curves from Siberia and south China. P, phosphogenic events.

archaeocyathans diversified explosively⁵. The latter were seldom associated with phosphatic shells or phosphorites, and a preference for oligotrophic conditions may be implied by analogy with recent reefal biotas plus evidence for close associations with cyanobacterial mounds. Symbiotic recycling of phosphorus, nitrogen and organic compounds is inferred.

The peak of archaeocyathan diversity coincided with maximum transgression during the early Cambrian, and declined during a eustatic regression⁵. A return to more phosphogenic conditions and heavier stable isotopes during and after this demise raises important questions about the role of nutrient excess in evolution, like that currently destroying coral reefs.

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1. Brasier, M.D. *et al.* *Geol. Mag.* **127**, 319–332 (1990).
2. Berger, W.H., Smetacek, V.S. & Wefer, G. *Productivity of the Ocean: Present and Past*. (Wiley, Chichester, 1989).
3. Droser, M. & Bottjer, D.J. *Abstr. 28th Int. geol. Congr., Washington* **1**, 418–419 (1989).
4. Cowie, J.W. & Brasier, M.D. (eds) *The Precambrian–Cambrian boundary*. (Oxford University Press, 1989).
5. Zhuraviev, A. *Geol. Mag.* **123**, 377–385 (1986).

Erratum

In the Scientific Correspondence "The law beats Maxwell's demon" by Paul A. Samuelson (*Nature* **347**, 24–25; 6 September 1990) the equation

$$D[\bar{x}, 1/2] = \bar{x} \text{ for } \bar{x} < 0$$

should have read

$$D[\bar{x}, 1/2] = -\bar{x} \text{ for } \bar{x} < 0.$$

□

HIV mutation rate

SIR—The human immunodeficiency virus (HIV) evolves at a rate about a million times as great as that of eukaryotic DNA genomes¹. This ratio is reflected in the replication accuracies of the corresponding enzymes: the typical error rate of DNA polymerization is somewhere around 10^{-10} and 10^{-9} ; the measured average error rate per site for the HIV-1 reverse transcriptase is between 10^{-4} and 10^{-3} (refs 2,3). This implies that the reverse transcriptase makes on average 1–10 errors during the replication of the HIV genome (10^4 bases). This high mutation rate might be an important factor for the virus to escape destruction by the immune system.

An interesting feature of HIV-1 infections is the high genetic variability found in virus populations. Sequential virus isolations from the same infected patients have shown that mutation *in vivo* is rapid: many related but distinguishable mutants evolve during chronic infection^{4–6}. The selection pressure exerted by the immune response might represent the main force to generate this genetic variability.

The existence of neutralizing antibodies against HIV has been well documented^{7,8}. The immunodominant loop (V3 loop), a region of about 30 amino acids within the viral envelope protein gp120, mediates most of the neutralization phenomena in infected humans. This part of the envelope protein seems to mutate rapidly in infected patients. Immunological selection (propagation of new resistant mutants in the presence of specific neutralizing agents) has been observed *in vitro*⁹. The generation of new neutralization resistant mutants might result from a single point mutation in the envelope gene¹⁰. This production of escape mutants by antigenic drift has also been reported for other lentiviruses.

It seems to be essential for the virus to change its immunodominant epitopes as fast as possible. An obvious question is: what is the optimal mutation rate that maximizes the probability to produce escape mutants?

If the average replication accuracy of the reverse transcriptase is denoted by q , the probability that replication of the whole genome is done without error is given by $Q = q^n$, where n is the total length of the genome. For HIV, $n \sim 10^4$ for one single strand. Let m be the number of sites within the genome such that a mutation in at least one of these sites results in the production of an escape mutant (m might be the length of the immunodominant loop or so, but the particular value for m will not influence our result, as long as m is much smaller than n , which is clear). The probability to obtain an escape mutant (the probability of obtaining a mutant with no errors in $n - m$ sites and at least

one error in the m sites) is given by $P = q^{n-m}(1 - q^m)$. This probability has a maximum for $q^* = (1 - m/n)^{1/m}$ which is well approximated by $q^* = 1 - 1/n$. This result is independent of m . Therefore, the optimal mutation rate is given by $e^* = 1 - q^* = 1/n$, which is for HIV around 10^{-4} .

This result could be shifted slightly to higher values if we consider the effect of neutral sites in the genome. Additional mutation in the neutral sites will not change the fitness of an escape mutant. If r is the fraction of neutral sites in the whole genome, then $P = q^{n(1-r)-m}(1 - q^m)$ and $e^* = 1/n(1-r)$. If r lies somewhere between 0 and 0.9, then e^* is found between 10^{-4} and 10^{-3} .

My argument is based on the assumption that the human immune response against HIV favours variation in some parts of the viral genome. If this is true, then there is an optimal mutation rate which maximizes the probability of producing new resistant mutants due to errors in viral replication. This optimal mutation rate is in good agreement with the measured replication accuracy of the HIV-1 reverse transcriptase.

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1. Hahn, B.H. *et al.* *Science* **232**, 1548–1553 (1986).
2. Preston, B.D. *et al.* *Science* **242**, 1168–1171 (1988).
3. Roberts, J.D. *et al.* *Science* **242**, 1171–1173 (1988).
4. Saag, M.S. *et al.* *Nature* **334**, 440–444 (1988).
5. Fisher, A.G. *et al.* *Nature* **334**, 444–447 (1988).
6. Wain-Hobson, S. *AIDS* **3**, (Suppl. 1) 513–518 (1989).
7. Robert-Guroff, M. *et al.* *Nature* **316**, 72 (1985).
8. Weiss, R.A. *et al.* *Nature* **316**, 69 (1985).
9. Robert-Guroff, M. *et al.* *J. Immun.* **137**, 3306–3309 (1986).
10. Looney, D.J. *et al.* *Science* **241**, 357–359 (1988).

Radiating bodies

SIR—H. Aspden (*Nature* **347**, 25; 1990) describes a procedure for driving sensible energy from a cooler body to a hotter body, in contravention of thermodynamics. This is done by the use of a mirror to focus radiation from the cooler body A onto the surface of the hotter body B.

This procedure will fail. If the only radiating bodies involved are the bodies A and B, then body B will radiate heat back to body A faster than A radiates to B, so bringing the two bodies to the same temperature, as required by thermodynamics. It would be necessary in practice to enclose both A and B by additional surfaces, but in order that these should make no net contributions to the energy exchanges between A and B, these too would have to be at the same temperature as A and B.

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Trace-element fractionation in plumes and the origin of HIMU mantle beneath the Cameroon line

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The lavas of the Cameroon line display a lead isotope anomaly at the continent/ocean boundary which can be attributed to a fossil mantle plume, the diminishing lateral effects of which can be recognized as far as 400 km to either side. The high $^{206}\text{Pb}/^{204}\text{Pb}$ ratio—characteristic of the mantle endmember known as 'HIMU'—seems to arise from U/Pb fractionation accompanying melt migration following emplacement of the plume in the upper mantle 125 Myr ago.

MODELS for the genesis of ocean island basalts (OIB) can generally be divided into those invoking direct partial melting of plumes that have risen from below a thermal boundary layer separating the lower mantle from the convecting upper mantle (the latter generally thought to be the source of mid-ocean ridge basalts (MORB))¹⁻³, and those invoking preferential melting of relatively enriched components which are heterogeneously dispersed through the convecting upper mantle⁴⁻⁷. Isotope variations in the mantle are also commonly modelled in terms of different proportions of endmember components. The most clearly defined of these is characterized by very radiogenic Pb and unradiogenic Nd relative to Sr and has been variously referred to as the 'St Helena type' by White⁸, the 'LoNd' type by Hart *et al.*⁹ and the 'HIMU' type by Zindler and Hart¹⁰. Magmas of this nature (called HIMU in this paper) have been recognized in St Helena¹¹, Tubuaii¹²⁻¹⁴, Mangaia^{12,13}, the Cameroon line^{15,16}, the Cape Verdes¹⁷ and the Oslo Rift¹⁸. The HIMU component possesses special significance because it also represents the most extreme case of the 'lead paradox'¹⁹ (the lead isotopic compositions of OIB and MORB imply that, relative to bulk Earth, their sources have time-integrated high U/Pb ratios, whereas a mantle source that has yielded melts should be characterized by low U/Pb because U is believed to be more incompatible than Pb). Explanations for the lead paradox generally, or for the HIMU component specifically, have included preferential partitioning of lead into the core for a significant period after accretion²⁰⁻²², recycling of ancient oceanic crust^{2,8,12,13,17,23}, delamination of sub-continental lithosphere^{9,24,25}, metasomatism by CO₂-rich fluids^{13,26}, preferential recycling back into the mantle of upper continental crust with the lower continental crust representing a repository of unradiogenic lead²⁷, and finally the possibility that lead is in fact more incompatible than uranium in mantle melting^{28,29}.

Studies of the volcanic rocks of the Cameroon line have played a useful part in constraining models for the genesis of OIB because of the unusual independent space-time constraints that can be applied to models of magma genesis^{7,15,30,31}. Here we

use these space-time constraints to demonstrate that HIMU characteristics were produced relatively recently in an upper-mantle plume as the time-integrated effect of trace-element fractionation between silicate minerals and melt, with U more incompatible than Pb.

The Cameroon line

The Cameroon line is a 1,600-km Y-shaped chain of volcanic centres and plutons extending northeast from the island of Pagalu in the Atlantic Ocean to the continental interior of west Africa (Fig. 1). There are 12 main volcanic centres, the products of which range in age from 30 Myr to the present⁷. In addition, there are 17 pluton complexes in the continental sector, which range in age from 65 to 30 Myr (refs 32, 33). It has been shown by isotope dating that there has been no systematic change in the focus of magmatism during this entire period⁷. Because the African plate has moved with respect to hotspots during the past 65 Myr (refs 34, 35), it is clear that the magmatic activity

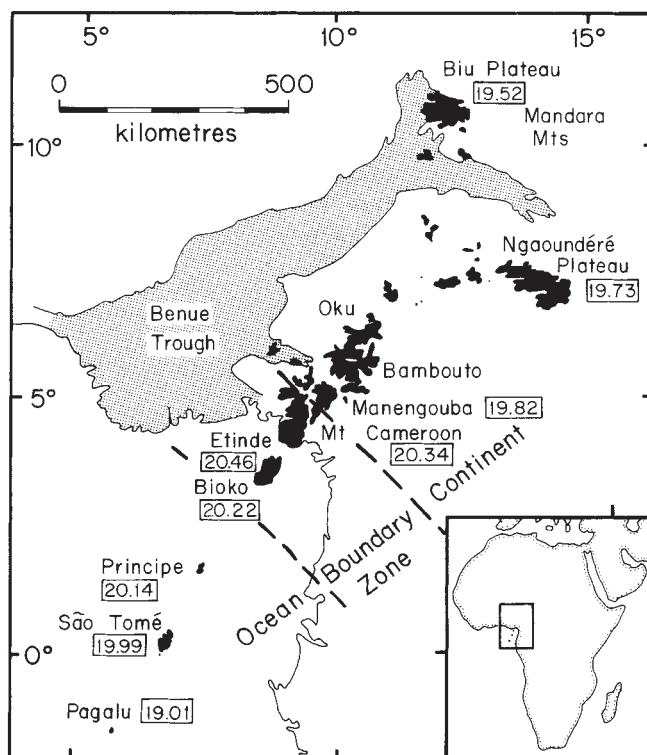


FIG. 1 Map of the area of the Cameroon line and the Gulf of Guinea showing the distribution of the main Cenozoic volcanic centres and the definition of the continent/ocean boundary region. The mean $^{206}\text{Pb}/^{204}\text{Pb}$ of the < 10 Myr lavas is given for each locality. Data from Table 1 and refs 15 and 16.

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TABLE 1 Isotope and selected chemical data

Volcanic centre	Sample number	Age (Myr)	Lithology	Rb (p.p.m.)	Sr (p.p.m.)	Nd (p.p.m.)	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{143}\text{Nd}/^{144}\text{Nd}$	ϵ_{Nd}	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{207}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{204}\text{Pb}$
Manengouba	C55	3	Hy-normative basalt	36	636	46	0.70316 (3)	0.512957 (7)	+6.2	19.794	15.609	39.36
Manengouba	C69	1	Trachybasalt	62	788	60	0.70315 (2)	0.512923 (8)	+5.6	19.709	15.613	39.35
Manengouba	C51	1	Alkali basalt	37	757	46	0.70308 (2)	0.512929 (31)	+5.7	19.920	15.611	39.52
Manengouba	C59	1	Alkali basalt	37	945	56	0.70307 (1)	0.512904 (10)	+5.2	20.135	15.665	39.87
Manengouba	C72	1	Alkali basalt	43	918	54	0.70303 (1)	0.512867 (20)	+4.5	20.182	15.694	39.87
Manengouba	C70	1	Basanite	42	733	43	0.70300 (1)	0.512940 (9)	+5.9	19.634	15.625	39.27
Manengouba	C56	1	Hy-normative basalt	16	480	27	0.70299 (1)	0.512959 (10)	+6.3	19.572	15.652	39.30
Manengouba	C68	1	Hy-normative basalt	38	463	38	0.70298 (2)	0.512986 (13)	+6.8	19.795	15.635	39.50
Mt Cameroon	C201	0	Basanite	22	661	42	0.70339 (1)	0.512723 (7)	+1.7	19.983	15.620	39.84
Mt Cameroon	C201D	0	Diopside megacryst	1	74	8	0.70336 (2)	0.512758 (9)	+2.3	20.110	15.640	39.61
Mt Cameroon	C25	0	Basanite	36	989	70	0.70335 (1)	0.512777 (7)	+2.7	20.354	15.658	40.15
Mt Cameroon	C5	0	Tephrite	51	1,202	81	0.70328 (1)	0.512781 (8)	+2.8	20.375	15.655	40.15
Etinde	C24	0	Nephelinite	87	2,401	180	0.70337 (1)	0.512782 (2)	+2.8	20.455	15.662	40.31
Etinde	C150	0	Melanephelinite	89	1,054	85	0.70337 (2)	0.512800 (6)	+3.2	20.289	15.642	40.08
Etinde	C131	0	Hauyne nephelinite	349	2,825	86	0.70334 (1)	0.512809 (7)	+3.3	20.470	15.665	40.28
Etinde	C154	0	Nosean leucite nephelinite	201	6,553	41	0.70333 (1)	0.512835 (7)	+3.8	20.522	15.663	40.34
Etinde	C152	0	Hauyne nephelinite	114	2,395	142	0.70331 (1)	0.512804 (8)	+3.2	20.489	15.666	40.31
Bioko	FP32	0	Tephrite	61	1,199	79	0.70343 (1)	0.512769 (8)	+2.6	20.368	15.667	40.21
Bioko	FP1	0	Alkali basalt	26	726	47	0.70325 (1)	0.512839 (8)	+3.9	20.032	15.652	39.86
Bioko	FP23	0	Alkali basalt	26	685	55	0.70323 (2)	0.512843 (8)	+4.0	20.298	15.691	40.05
Bioko	FP38	0	Alkali basalt	25	647	43	0.70319 (1)	0.512860 (7)	+4.3	20.044	15.646	39.80
Principe	P16	6	Trachyphonolite	314	11	17	0.71092 (2)	0.512815 (9)	+3.5	20.224	15.674	39.80
Principe	P39	6	Phonolite	271	79	17	0.70378 (2)	0.512940 (8)	+5.9	20.259	15.677	39.80
Principe	P40	6	Phonolite	216	240	23	0.70328 (1)	0.512906 (8)	+5.2	20.285	15.673	39.82
Principe	P12	6	Tristanite	180	1,115	36	0.70322 (1)	0.512922 (10)	+5.5	20.226	15.680	39.86
Principe	P13	6	Tristanite	185	1,188	37	0.70301 (2)	0.512865 (20)	+4.4	20.237	15.701	39.93
Principe	P41	6	Trachyphonolite	174	484	27	0.70300 (1)	0.512930 (7)	+5.7	20.190	15.684	39.82
São Tomé	ST54	3	Trachyphonolite	213	468	20	0.70332 (2)	0.512853 (30)	+4.2	20.128	15.674	39.87
São Tomé	ST93	3	Hy-normative basalt	8	1,174	87	0.70320 (1)	0.512889 (10)	+4.9	20.064	15.666	39.79
São Tomé	ST72	3	Basanite	46	1,130	67	0.70318 (1)	0.512919 (6)	+5.5	19.997	15.683	39.72
São Tomé	ST43	1.3	Trachyte	129	463	46	0.70316 (1)	0.512937 (7)	+5.8	20.060	15.658	39.69
São Tomé	ST106	3	Alkali basalt	29	836	51	0.70315 (2)	0.512899 (21)	+5.1	19.944	15.653	39.56
São Tomé	ST100	3	Trachybasalt	139	1,204	39	0.70312 (1)	0.512925 (9)	+5.6	20.014	15.659	39.68
São Tomé	ST73	3	Tephrite	64	1,281	79	0.70307 (1)	0.512953 (7)	+6.1	20.074	15.720	39.80
São Tomé	ST110	3	Phonolitic tephrite	114	1,128	52	0.70299 (1)	0.512980 (10)	+6.7	20.124	15.691	39.84
São Tomé	ST109	3	Basanite	29	824	44	0.70299 (2)	0.512998 (7)	+7.0	19.954	15.676	39.57

Concentrations determined by X-ray fluorescence at University of Edinburgh. Isotope data determined at the Radiogenic Isotope Geochemistry Laboratory (University of Michigan) using techniques outlined elsewhere⁵⁵. Errors refer to least significant digits and are 2σ mean run precisions. All isotopic ratios measured using a VG Sector multicollector mass spectrometer. Nd and Sr isotopic compositions were measured by multi-dynamic peak switching. Pb isotope data determined using static multicollecion except C201D, measured with a Daly detector and ion counting. We obtain $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.710242 (8) (2σ mean, $N=13$) for NBS987, and $^{143}\text{Nd}/^{144}\text{Nd}$ of 0.511855 (8) ($N=18$) for the La Jolla Nd standard. $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are normalized to $^{86}\text{Sr}/^{88}\text{Sr}=0.1194$, and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios are normalized to $^{146}\text{Nd}/^{144}\text{Nd}=0.7219$ with power-law fractionation corrections. Pb isotope data are corrected for fractionation and discrimination with a factor of 0.101% per a.m.u., based on measurements of the NBS981 standard, $\epsilon_{\text{Nd}} = \{[(^{143}\text{Nd}/^{144}\text{Nd})_{\text{sample}}/(^{143}\text{Nd}/^{144}\text{Nd})_{\text{bulk Earth}}] - 1\} \times 10^4$. Bulk Earth $^{143}\text{Nd}/^{144}\text{Nd}=0.512638$, $^{147}\text{Sm}/^{144}\text{Nd}=0.1966$.

cannot be explained by conventional plume models (as have been advanced³⁶ to explain the Hawaiian hotspot trail, for example). Fitton and co-workers showed^{7,30} that the average trace-element compositions of basalts in the Cameroon line were the same in continental and oceanic sectors, and argued that they were therefore derived from sub-lithospheric depths. A recent isotope study revealed, however, that lavas from the continent/ocean boundary region had relatively radiogenic lead, implying a genetic link with the lithosphere¹⁵.

The isotope anomaly

Table 1 shows Nd, Sr and Pb isotope data for young (<10 Myr) samples of basalt, basanite, nephelinite and more evolved rocks from volcanic centres close to the continent/ocean boundary. The three volcanic centres at the continent/ocean boundary (Bioko, Etinde and Mt Cameroon) are distinctive, with radiogenic Pb and unradiogenic Nd for a given Sr isotopic composition relative to the 'mantle array' (Figs 2 and 3). In these respects the data are similar to the compositions observed for Cape Verde, St Helena, Mangaia and Tubuai^{12–14,17}, and therefore of the HIMU mantle type¹⁰. The anomaly straddles the continent/ocean boundary and is therefore not lithospheric contamination in the normal sense. This is supported by the data for diopside megacryst C201D and its host lava (C201), which have the least radiogenic Pb and Nd of the Mt Cameroon samples (Table 1). Slight contamination by old lithospheric components is a likely cause of such an effect (and for this reason these samples are ignored in further discussion) but clearly could not explain the overall very radiogenic Pb of the

continent/ocean boundary lavas. Although the Pb isotope anomaly is most apparent in the three continent/ocean boundary volcanic centres, its effect can be observed in a progressively decreasing manner in the mean Pb isotopic compositions of younger lavas to either side of this zone (Fig. 1). Older Cameroon line lavas at a given locality tend to have less radiogenic lead¹⁵. Therefore to demonstrate clearly the magnitude of the geographical effect only analyses of young (<10 Myr) lavas are included in Table 1 and Fig. 1.

Plume models

The existence of a lead isotope anomaly in young lavas associated with an older lithospheric boundary underlines the inadequacy of conventional plume models for the Cameroon line (as with many rift-related magmatic provinces³⁷) because the geometrical coincidence between a plume from the lower mantle and the continent/ocean boundary, which has moved with plate motions, would have to be accidental. A plausible explanation for the isotope anomaly is that the chemical and isotope characteristics of the magmas are inherited from the melting and/or assimilation of plume-modified mantle that has remained spatially fixed relative to the lithosphere since the formation of the Gulf of Guinea¹⁵. This mantle would have been a mixture of asthenospheric mantle, excavated older sub-continental lithosphere, and components from a newly rising plume, which had already been instrumental in the formation of the continent/ocean boundary.

At the time of opening of the south Atlantic, the region occupied by the present continent/ocean boundary in the vicin-

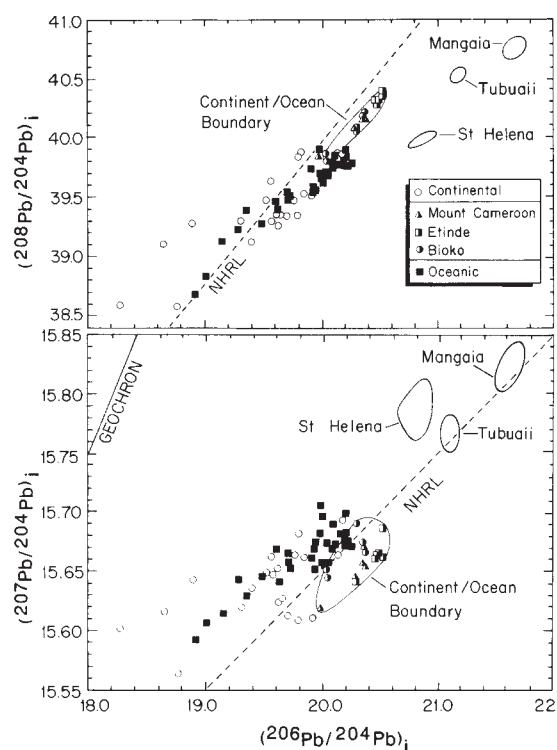


FIG. 2 Plot of initial Pb isotope data for the volcanic rocks of the Cameroon line, in the context of other HIMU (LoNd) types. Data from Table 1, refs 15, 16 and unpublished data for older lavas.

ity of the Gulf of Guinea (Fig. 1) overlay the St Helena hotspot³⁵. The idea that the St Helena plume was instrumental in the breakup of Africa, the transfer of HIMU components into the upper mantle and the resultant location of a (diluted) Pb isotope anomaly along the Cameroon line is therefore appealing¹⁵. The data presented here indicate that such a plume affected the surrounding mantle over a radius of some 400 km. This is similar to the size of plume heads envisaged by White and McKenzie³⁸.

Schilling and co-workers^{39,40} have demonstrated the lateral effects of hotspots on the Mid-Atlantic Ridge and attributed such effects to large-scale lateral flow towards the ridge. In the case of the Cameroon line it is clear that unless the relationship between the hotspots and the continent/ocean boundary is accidental rather than genetic, the general symmetry and apex of the hypothesized plume has been preserved in its original location in the uppermost mantle for 125 Myr. The Cameroon line data cannot be explained by models of redistribution of heterogeneities by large-scale lateral flow in the asthenosphere.

The generation of plume-like anomalies

Despite the apparent elegance of a fossil-plume model and the general similarities between St Helena and Cameroon isotopic compositions, there are problems with this interpretation. The Pb isotope data for the St Helena volcanic rocks are in fact distinct from those of the Cameroon line, and the differences cannot easily be reconciled by hypothesizing different proportions of the same plume component mixed with other Cameroon line compositions. The St Helena volcanic rocks display lower $^{208}\text{Pb}/^{204}\text{Pb}$ and higher $^{207}\text{Pb}/^{204}\text{Pb}$ (Fig. 2). The κ_{Pb} values (the time-integrated Th/U ratios deduced from the Pb isotope data⁴¹) for the continent/ocean boundary samples are consistently 3.9 to 4.0, compared with the values of ~ 3.7 for St Helena⁴¹. Furthermore, although showing the same effect of displacement below the 'mantle array', the Nd and Sr isotopic compositions do not lie on a mixing trajectory from the other Cameroon line data towards the field for St Helena (Fig. 3).

A significant feature of the lead isotope data for the continent/ocean boundary samples is that the range in $^{207}\text{Pb}/^{204}\text{Pb}$ is relatively uniform between different volcanic centres and displays no special distinction from the data for the rest of the Cameroon line; it is only the $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ ratios that are especially high (Fig. 2). This is illustrated in Fig. 4 where the different centres can be seen to define a series of sub-parallel arrays on a plot of $^{207}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$. The slopes of these arrays correspond to apparent ages of roughly 1 Gyr. Such parallel arrays cannot be easily explained as offsets produced by different degrees of mixing between two heterogeneous components with a similar range in $^{207}\text{Pb}/^{204}\text{Pb}$ (although clearly this could have enhanced the scatter) because each volcanic centre seems to be relatively restricted in its range of $^{206}\text{Pb}/^{204}\text{Pb}$. Neither can they represent a variety of mixing lines between a radiogenic lead component with high $^{207}\text{Pb}/^{204}\text{Pb}$ and a range of different components with less radiogenic and variable $^{206}\text{Pb}/^{204}\text{Pb}$ ratios because the plot of $^{208}\text{Pb}/^{204}\text{Pb}$ against $^{206}\text{Pb}/^{204}\text{Pb}$ displays a tight collinear trend for virtually all of the Cameroon line samples (Fig. 2a). The offsets between different volcanic centres displayed in Fig. 4 are most simply explained as the result of relatively recent variable enrichment in the U/Pb and Th/Pb ratios. This has the effect of producing correlated offsets in $^{206}\text{Pb}/^{204}\text{Pb}$ and $^{208}\text{Pb}/^{204}\text{Pb}$, with little change in $^{207}\text{Pb}/^{204}\text{Pb}$, because of the much longer half-lives of ^{238}U and ^{232}Th relative to ^{235}U . Such a process is easily modelled as shown in Fig. 4, assuming that the last major fractionation event took place at 125 Myr when the Gulf of Guinea (hence continent/ocean boundary) formed. The lead isotope data themselves merely indicate young trace-element fractionation; it is the coincidence and inferred genetic link with the continent/ocean boundary that form the basis for proposing the exact timing as 125 Myr. The general magnitudes of $^{238}\text{U}/^{204}\text{Pb}$ and $^{232}\text{Th}/^{238}\text{U}$ (approximately equal to Th/U) ratios required to generate the range of modelled lead isotopic compositions are also indicated on Fig. 4. How realistic these values are can be assessed in part by comparisons with measured U/Pb ratios for the lavas themselves. Table 2 provides new isotope-dilution data for U, Pb, Sm and Nd in lavas from the three continent/ocean boundary volcanic centres. The average measured $^{238}\text{U}/^{204}\text{Pb}$ ratios are generally similar to the modelled values in Fig. 4 and they increase in the order Bioko, Mt Cameroon, Etinde, as predicted by the model.

Implicit in this model is the generally held assumption (recently challenged by Meijer and co-workers^{28,29} who have argued the opposite as an explanation for the lead paradox)

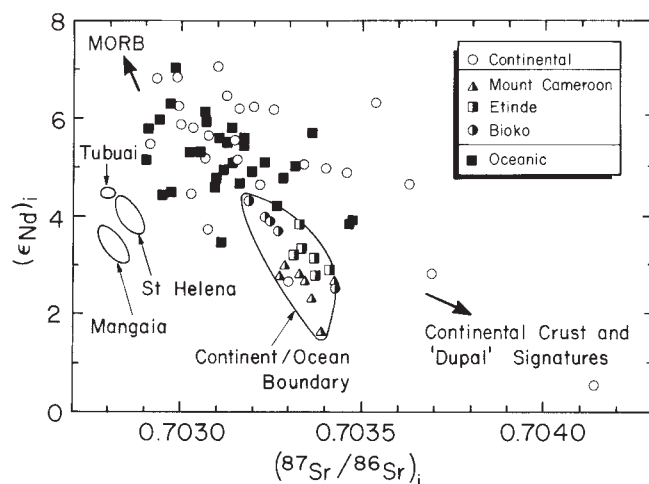


FIG. 3 Plot of initial ϵ_{Nd} against initial $^{87}\text{Sr}/^{86}\text{Sr}$ for volcanic rocks of the Cameroon line, in the context of other HIMU (LoNd) types. Data from Table 1, refs 15, 16 and unpublished data for older lavas.

TABLE 2 Sm, Nd, U and Pb concentrations

Locality	Sample number	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}$	U (p.p.m.)	Pb (p.p.m.)	$^{238}\text{U}/^{204}\text{Pb}$
Bioko	FP1	9.67	45.6	0.1282	1.07	2.76	25.7
	FP38	8.79	40.5	0.1311	0.775	2.54	20.2
Etiende	C24	25.1	17.6	0.08613	5.68	7.66	49.8
	C150	14.3	89.6	0.09662	2.45	3.84	42.6
Mount Cameroon	C201	7.72	43.0	0.1085	1.25	2.41	34.3
	C25	11.9	68.9	0.1043	2.13	3.75	38.0

Concentrations determined by isotope dilution.

that U is a more incompatible element than Pb in mantle melting. This can be substantiated using the Cameroon line data. Figure 5a shows that there is a general positive correlation between lead isotopic composition and measured $^{238}\text{U}/^{204}\text{Pb}$ ratio of the more primitive Cameroon line basalts, the slope of which is

consistent with a model of young growth of radiogenic lead in the mantle. This relationship suggests that the $^{238}\text{U}/^{204}\text{Pb}$ ratio of the lavas is a function of the ratio in the source. Figure 5b, c shows that the U concentration increases, and the Sm/Nd ratio decreases, with increasing U/Pb ratio, consistent with U being more incompatible than Pb in these magmas. The simplest explanation for a relationship between U/Pb, Nd/Sm and $^{206}\text{Pb}/^{204}\text{Pb}$ ratio is that these ratios are correlated in the sources of the magmas. These data, together with those for other basalts, support the view⁴² that U is more incompatible than Pb in mantle melting and that the fractionation in U/Pb ratios (as with Sm/Nd ratios) is dominantly controlled by silicate mineral/melt equilibria, rather than by sulphide or metal phases (see ref. 29).

A consequence of the inverse relationship between U/Pb and Sm/Nd fractionation is that increased source U/Pb ratios toward the continent/ocean boundary should be accompanied by decreased Sm/Nd ratios and therefore relatively unradiogenic Nd as shown in Fig. 3. Figure 4 shows the relationship between $^{206}\text{Pb}/^{204}\text{Pb}$ ratios and the Nd isotopic compositions of the young volcanic rocks of the Cameroon line. The general trends of the data are consistent with our model, but for the variations in Nd and Sr isotopic composition to be generated entirely by radioactive decay over 125 Myr requires source variations of 0.2 in both $^{147}\text{Sm}/^{144}\text{Nd}$ and $^{87}\text{Rb}/^{86}\text{Sr}$. Given the range in U/Pb, Rb/Sr and Sm/Nd measured in the spectrum of OIB and pristine MORB glasses^{15,42,43}, the modelled source parent/daughter ratios are not impossible, although extreme, particularly for Sm/Nd. The most likely explanation for this is that the lavas from farther away from the continent/ocean boundary have inherited components from more depleted mantle with high Nd/Pb and Sr/Pb ratios such as MORB sources. This explanation is supported by the modelled curvature of the data arrays toward a depleted mantle component with high Nd/Pb (Fig. 4). Such a model is further supported by the relatively unradiogenic lead isotopic compositions of the more distant Cameroon line volcanic centres of Pagalu, Biu and Ngaoundéré¹⁵.

Plume differentiation and interaction

Recent attempts to model the fluid dynamics of plumes and thermals⁴⁴⁻⁴⁸ have shown that such bodies may rise by a combination of shear flow and entrainment of the surrounding mantle, resulting in incorporation and circulation of components from the ambient mantle in the plume head itself. As a result, the compositional outline of the plume head may develop a 'doughnut shape', with a core of contaminated material. This has the interesting effect that the mantle in the core of the head should not be as enriched as that occupying the region immediately to either side (assuming that the plume is trace-element enriched relative to the ambient mantle)—which should produce a widely spaced pair of anomalies to either side of the plume axis, quite unlike the pattern shown in Fig. 1. We propose instead that the magmas are derived by remelting of a fossil plume, and that the isotope anomaly observed towards the central portions of the Cameroon line, reflects the time-integrated effect of trace-element fractionation during melt migration in the plume head as it cooled after emplacement at 125 Myr. We suggest that the Cameroon line magmas are currently derived from a zone in

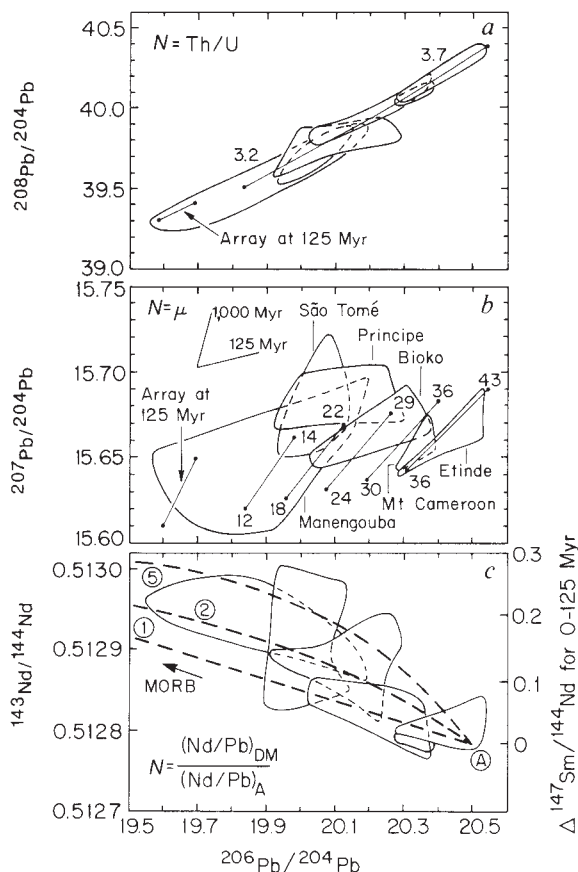


FIG. 4 Pb and Nd isotope data for young (<10 Myr) lavas of the Cameroon line divided according to volcanic centres. The Pb isotope differences between the volcanic centres are explicable if there has been relatively recent fractionation of the U/Pb ratios which will have a greater effect on the $^{206}\text{Pb}/^{204}\text{Pb}$ ratio than the $^{207}\text{Pb}/^{204}\text{Pb}$ ratio. The lines in a and b are model Pb isotope arrays produced from an initial linear array at 125 Myr (shown at the left) using the different $^{238}\text{U}/^{204}\text{Pb}$ and $^{232}\text{Th}/^{238}\text{U}$ values indicated. The model assumes that the $^{238}\text{U}/^{204}\text{Pb}$ and $^{232}\text{Th}/^{238}\text{U}$ values are proportional to each other and to variations in the $^{238}\text{U}/^{204}\text{Pb}$ and $^{232}\text{Th}/^{238}\text{U}$ values that produced the original array. c shows the relationship between the Pb and Nd isotopic compositions and the considerable $^{147}\text{Sm}/^{144}\text{Nd}$ variation necessary to account for the Nd isotope variations purely by radioactive decay over the past 125 Myr. An explanation for the large variation in Nd isotopic composition is that the magmas farther away from the continent/ocean boundary include components from depleted (MORB-like) sources. This is modelled with mixing curves constructed for different values of N (circled). Data from Table 1, and refs 15 and 16.

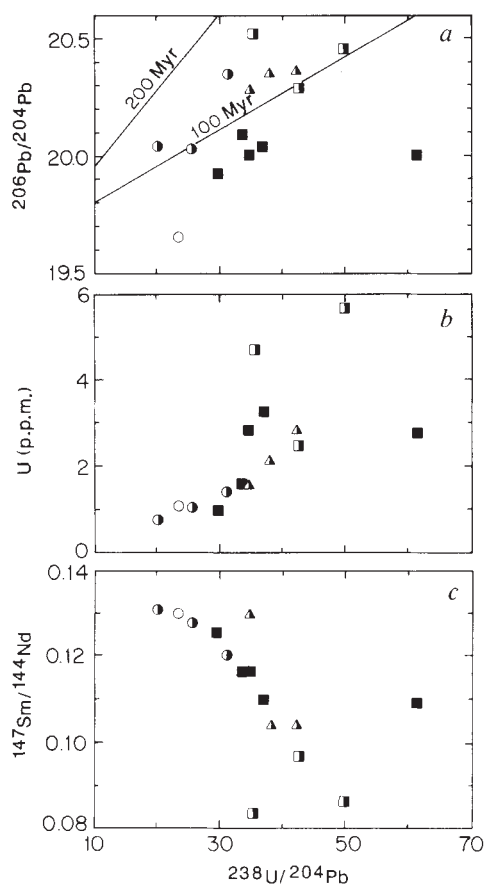


FIG. 5 Relationship between the $^{238}\text{U}/^{204}\text{Pb}$ and a, $^{206}\text{Pb}/^{204}\text{Pb}$, b, U concentration and c, $^{147}\text{Sm}/^{144}\text{Nd}$ for the young (<10 Myr) Cameroon line lavas with >4 wt% MgO from the volcanic centres in Table 1. These relationships are easily explained if U is more incompatible than Pb and U/Pb (like Sm/Nd) is controlled by silicate solid/liquid partitioning in the magma sources. The one oceanic sample with anomalously high $^{238}\text{U}/^{204}\text{Pb}$ is a nephelinite from Principe. Data from Tables 1 and 2, and refs 15, 16.

the upper portions of the fossil plume, enriched by basaltic melts that percolated upward during compaction of the head (Fig. 6). The degree of trace-element enrichment (hence U/Pb ratio) in this zone, would then increase laterally towards the centre, as a function of the vertical thickness of relatively enriched mantle (the flattened plume head) through which the melts migrated during plume compaction. At the margins, where the flattened plume is thinnest, the source regions are dominated by more depleted mantle (Fig. 6), as reflected in the change in Nd, Sr and Pb isotopic compositions towards either end of the Cameroon line, culminating in the highly depleted isotopic compositions observed for centres like Pagalu.

Trace-element fractionation

The Pb isotopic compositions for the three continent/ocean boundary centres imply source U/Pb minima of 0.152 for Etinde and 0.033 for Mt Cameroon, based on the differences with Bioko, assuming closed-system evolution over 125 Myr. The Th/U ratio of the source of these three volcanoes can be estimated at ~3.3 (from the slope of the data in Fig. 2a). Although this is probably a slight overestimate⁴⁹ it is consistent with the modelling in Fig. 4, and is intermediate between the Cameroon line κ_{Pb} values (3.9–4.0) and the κ_{Th} values of MORB (2.5) (ref. 41). These U/Pb and Th/U estimates, together with the model estimates shown in Fig. 4, provide evidence that the upper-mantle sources are more enriched than MORB sources (in contrast to some models for OIB⁷).

The minimum U/Pb ratio measured for the lavas can be compared with that estimated for the sources based on time-integrated differences, to derive a value for the maximum fractionation in U/Pb ratio (4) during production of the continent/ocean boundary magmas. The U/Pb fractionation required to generate the range in lead isotopic compositions is less than five. U/Pb fractionations in this range can easily be achieved with fractional melting^{50,51}, and bulk (solid/liquid) distribution coefficients for U and Pb that are within a factor of about six of each other, assuming a constant melt fraction in contact with solid of 0.01, and fractions of melt removed of between 0.01 and 0.10. The U/Pb fractionations hypothesized for both 125 Myr and during the melting that produced the young Cameroon line magmas, are therefore reasonable given the range of experimental silicate mineral/melt U and Pb partition coefficients^{52,53}, and do not require special CO_2 metasomatism or phases other than silicates.

The reasonably common occurrence of basalts with HIMU isotope characteristics is evidence that the fractionation processes that took place during melting in the mantle under the Cameroon line may not be unusual. Obviously such fractionations do not have to be recent as in the case of the Cameroon line (the St Helena $^{207}\text{Pb}/^{204}\text{Pb}$ ratios cannot be explained by the model of recent fractionation presented here). Nevertheless an important implication of the large range in parent/daughter ratios measured and modelled here is that they will generate mantle that is extremely heterogeneous isotopically if allowed to evolve over long periods of time (gigayears). Although such an effect is well illustrated by storage in the sub-continental lithosphere⁵⁴, the relative homogeneity of MORB and to a lesser extent OIB compositions⁴³ implies that heterogeneities produced from small amounts of partial melting are destroyed or greatly diluted in the long-term cycling of the convecting mantle. In these respects our data are consistent with the evidence for short residence times for lead in the upper mantle presented by Galer and O'Nions⁴¹.

Although the Cameroon line plume was apparently already relatively enriched when it was introduced into the upper mantle at 125 Myr, the high κ_{Pb} values for the Cameroon line are difficult to reconcile with models that explain this enrichment in terms of recycling of uranium-enriched (low Th/U) altered oceanic crust into the deep mantle (such as is more readily applied to the data for St Helena, for example). If trace-element fractionation by melting in the mantle produces Nd–Sr isotope data that are below the mantle array and can be taken to the

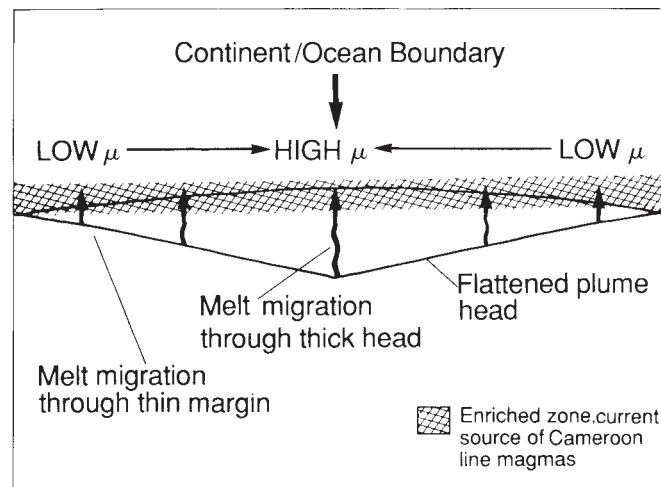


FIG. 6 Cartoon illustrating model for source enrichment following plume emplacement at 125 Myr.

extreme of explaining all HIMU components, the case for a distinctive isotope signature for ancient recycled oceanic crust may need re-evaluating. Conversely the best explanation for the mantle array itself may be recycling, rather than melt fractionation^{10,11}. Regardless of these possibilities, this study shows that

HIMU mantle can simply represent the time-integrated consequence of storage of small-degree partial melts in the upper mantle and, contrary to recent suggestions, the lead paradox is not resolvable simply in terms of U and Pb partitioning during melting. □

Received 9 July; accepted 23 August 1990.

1. Allègre, C. J. *Tectonophysics* **81**, 109–132 (1982).
2. Hofmann, A. W. & White, W. M. *Earth planet. Sci. Lett.* **57**, 421–436 (1982).
3. Storey, M. et al. *Nature* **336**, 371–374 (1988).
4. Morris, J. D. & Hart, S. R. *Geochim. cosmochim. Acta* **47**, 2015–2030 (1983).
5. Sleep, N. H. *J. geophys. Res.* **89**, 10029–10041 (1984).
6. Zindler, A., Staudigel, H. & Batiza, R. *Earth planet. Sci. Lett.* **70**, 175–195 (1984).
7. Fitton, J. G. & Dunlop, H. M. *Earth planet. Sci. Lett.* **72**, 23–38 (1985).
8. White, W. M. *Geology* **13**, 115–118 (1985).
9. Hart, S. R., Gerlach, D. C. & White, W. M. *Geochim. cosmochim. Acta* **50**, 1551–1557 (1986).
10. Zindler, A. & Hart, S. A. *Rev. Earth planet. Sci.* **14**, 493–571 (1986).
11. White, W. M. & Hofmann, A. W. *Nature* **196**, 821–825 (1982).
12. Palacz, Z. A. & Saunders, A. D. *Earth planet. Sci. Lett.* **79**, 270–280 (1986).
13. Nakamura, Y. & Tatsumoto, M. *Geochim. cosmochim. Acta* **52**, 2909–2924 (1988).
14. Vidal, Ph., Chauvel, C. & Brousse, R. *Nature* **307**, 536–538 (1984).
15. Halliday, A. N., Dickinson, A. P., Fallick, A. E. & Fitton, J. B. *J. Petrol.* **29**, 181–211 (1988).
16. Fitton, J. G., Kilburn, C. R. J., Thirlwall, M. F. & Hughes, D. J. *Nature* **306**, 327–332 (1983).
17. Gerlach, D. C., Cliff, R. A., Davies, G. R., Norry, M. & Hodgson, N. *Geochim. cosmochim. Acta* **52**, 2979–2992 (1988).
18. Anthony, E. Y., Segalstad, T. V. & Neuman, E. R. *Geochim. cosmochim. Acta* **53**, 1067–1076 (1989).
19. Allègre, C. J. *Earth planet. Sci. Lett.* **5**, 261–269 (1969).
20. Vidal, P. & Dosso, L. *Geophys. Res. Lett.* **5**, 169–172 (1978).
21. Vollmer, R. *Nature* **270**, 144–147 (1977).
22. Allègre, C. J., Dupre, B. & Brevart, O. *Phil. Trans. R. Soc. A* **306**, 49–59 (1982).
23. Chase, C. G. *Earth planet. Sci. Lett.* **52**, 277–284 (1981).
24. McKenzie, D. & O'Nions, R. K. *Nature* **301**, 229–231 (1983).
25. O'Nions, R. K. *J. geol. Soc. Lond.* **144**, 259–274 (1987).
26. Menzies, M. A. & Wass, S. Y. *Earth planet. Sci. Lett.* **65**, 287–302 (1983).
27. O'Nions, R. K., Evensen, N. M. & Hamilton, P. J. *J. geophys. Res.* **84**, 6091–6101 (1979).
28. Meijer, A. *Geophys. Res. Lett.* **12**, 741–744 (1985).
29. Meijer, A., Kwon, T.-T. & Tilton, G. R. *J. geophys. Res.* **95**, 433–448 (1990).
30. Fitton, J. G. *Spec. Publs. geol. Soc. Lond.* **30**, 273–291 (1987).
31. Dunlop, H. M. & Fitton, J. G. *Contr. Miner. Petrol.* **71**, 125–131 (1979).
32. Lasserre, M. *Bull. Bur. Rech. geol. min. Paris 2e Ser., Sect. IV, No. 2*, 143–159 (1978).
33. Cantagrel, J.-M., Jamond, C. & Lasserre, M. *C. r. somm. Soc. geol. Fr.* **6**, 300–303 (1978).
34. Morgan, J. W. *Tectonophysics* **94**, 123–139 (1983).
35. Fitton, J. G. *Earth planet. Sci. Lett.* **51**, 132–138 (1980).
36. Dairymple, G. B., Clague, D. A., Garcia, M. O. & Bright, S. W. *Bull. geol. Soc. Am.* **92**, 315–318 (1981).
37. Phipps, S. P. *Nature* **334**, 27–31 (1988).
38. White, R. & McKenzie, D. *J. geophys. Res.* **94**, 7685–7729 (1989).
39. Schilling, J.-G. *Nature* **314**, 62–67 (1985).
40. Schilling, J.-G., Thompson, G., Kingsley, R. & Humphris, S. *Nature* **313**, 187–191 (1985).
41. Gaier, S. J. G. & O'Nions, R. K. *Nature* **316**, 778–782 (1985).
42. Newsom, H. E., White, W. M., Jochum, K. P. & Hofmann, A. W. *Earth planet. Sci. Lett.* **80**, 299–313 (1986).
43. Cohen, R. S. & O'Nions, R. K. *J. Petrol.* **23**, 299–324 (1982).
44. Griffiths, R. W., Gurnis, M. & Eitelberg, G. *Geophys. J.* **96**, 477–495 (1989).
45. Olson, P. & Singer, H. *J. Fluid Mech.* **158**, 511–531 (1985).
46. Griffiths, R. W. *Earth planet. Sci. Lett.* **78**, 435–446 (1986).
47. Griffiths, R. W. *Phys. Earth planet. Inter.* **43**, 261–273 (1986).
48. Griffiths, R. W. *J. Fluid Mech.* **166**, 115–138 (1986).
49. White, W. M., Hofmann, A. W. & Puchelt, H. *J. geophys. Res.* **92**, 4881–4893 (1987).
50. McKenzie, D. *Earth planet. Sci. Lett.* **74**, 81–91 (1985).
51. Langmuir, C. H., Bender, J. F., Bence, A. E. & Hanson, G. N. *Earth planet. Sci. Lett.* **36**, 133–156 (1977).
52. Seitz, M. G. *Yb. Carnegie Instn. Wash.* **72**, 551–553 (1973).
53. Watson, E. B., Ben Othman, D., Luck, J.-M. & Hofmann, A. W. *Chem. Geol.* **62**, 191–208 (1987).
54. Vollmer, R. & Norry, M. J. *Nature* **301**, 141–143 (1983).
55. Halliday, A. N. et al. *Earth planet. Sci. Lett.* **94**, 274–290 (1989).

ACKNOWLEDGEMENTS. We thank R. Keller and M. Johnson for technical help, S. Djallo, R. Temdijm, G. Davies, M. Gurnis and K. Mezger for discussions, and W. White and A. Saunders for review. This work was supported by the Turner Fund (University of Michigan) and the NSF (A.N.H.) and by NERC (J.G.F.).

Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *pit-1*

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Mutations at the mouse dwarf locus (*dw*) interrupt the normal development of the anterior pituitary gland, resulting in the loss of expression of growth hormone, prolactin and thyroid-stimulating hormone, and hypoplasia of their respective cell types. Disruptions in the gene encoding the POU-domain transcription factor, *Pit-1*, occur in both characterized alleles of the dwarf locus. The data indicate that *Pit-1* is necessary for the specification of the phenotype of three cell types in the anterior pituitary, and directly link a transcription factor to commitment and progression events in mammalian organogenesis.

ANALYSES of developmental *Drosophila* and *Caenorhabditis elegans* mutants^{1–4} suggest models in which sequential activation of a hierarchy of regulatory genes dictates the temporal and

spatial patterns of the expression of gene products that define cell phenotypes. Similarly, organogenesis in mammals results from the ordered expression of cell phenotypes and, presumably, is also determined by the sequential activation of regulatory genes. The development of the anterior pituitary gland provides an excellent model system in which to study cell-specific gene activation and the processes by which distinct cell types develop within an organ. The anterior pituitary arises from a placode in Rathke's pouch, derived from the somatic ectoderm⁵. The five phenotypically distinct cell types appear in a stereotypical order with the growth hormone-producing somatotrophs and the prolactin-producing lactotrophs being the last two cell types to appear^{1–7}.

The cell-specific expression of the rat prolactin and growth hormone genes depends on synergistic interactions between *cis*-active elements^{8–16}. A pituitary-specific transcription factor, *Pit-1*, binds to these elements in both genes and can transactivate prolactin and growth hormone gene promoters both *in vitro* and in cell transfection experiments (refs 17–20, R. Maurer & D. Sharp personal communication), although one group²¹ found that *Pit-1* binds only to the growth hormone gene promoter. The

expression of *pit-1* is initially detected on embryonic days 15 and 16 (e15–16) in the rat, followed by the expression of growth hormone and prolactin on embryonic day 17 (ref. 7). Because *pit-1* expression precedes the expression of prolactin and growth hormone genes, the ontogeny of expression of the gene encoding Pit-1 is consistent with a critical role for this transcription factor in the expression of prolactin and growth hormone genes. In the mature pituitary, Pit-1 protein is detected in somatotrophs, lactotrophs and thyrotrophs⁷. Pit-1 is a member of a large gene family of transcription factors, referred to as POU-domain proteins, characterized by a 60-amino-acid region similar to the classical homeodomain and a 76-amino acid region, referred to as the POU-specific domain (for review see refs 22–24). In *C. elegans*, the POU-domain gene *unc-86* regulates developmental events²⁵. These data implicate the POU-domain transcription factors in the regulation of cell-specific developmental events in mammals.

Because a mutation in the *pit-1* gene would be predicted to abolish the expression of growth hormone, this mutation would most probably cause a dwarf phenotype. The Snell dwarf mouse (*dw*) was the first genetically transmitted dwarfism observed in mice, and was characterized as a single autosomal recessive mutation^{26,27}. The primary defect in *dw* was first suggested to be intrinsic to the anterior pituitary gland because of the hypoplastic nature of the organ^{28,29} and the results of transplantation experiments³⁰. Antisera against growth hormone, prolactin and thyroid-stimulating hormone (TSH) failed to detect synthesis of these hormones in the anterior pituitary, and the mature thyrotroph, lactotroph and somatotroph cell types were not observed in Snell dwarfs by electron microscopy^{31–34}. The mutation causing the dwarf Jackson (*dw*^J) mouse was discovered on a C3H/HeJ inbred background, and is allelic to the Snell dwarf mutation³⁵. A nonallelic but phenotypically similar dwarf mouse mutation called Ames dwarf (*df*) defines a second dwarf locus^{36,37}. The phenotype of both dwarf loci includes the depletion of somatotroph, lactotroph and thyrotroph cell types in homozygotes^{31–34}. Several observations suggest the possibility that defects in the *pit-1* gene might result in a dwarf phenotype,

including the ontogeny of *pit-1* expression, its expression in the three pituitary cell types that are affected in dwarf mutants, and the capacity of Pit-1 to activate the growth hormone and prolactin gene promoters.

Here we demonstrate a gross structural alteration of the *pit-1* gene in the dwarf Jackson mouse, and a point mutation of an invariant residue in the POU-homeodomain 'recognition helix' that generates a nonfunctional Pit-1 protein in the Snell dwarf. Pit-1 transcripts or protein were not detectable by *in situ* hybridization or immunohistochemistry for mutants with either allele of the *dw* locus.

Location of *pit-1* and the *dw* locus

The *pit-1* gene could be the candidate for either *dw* locus on chromosome 16 (ref. 35) or the *df* locus on chromosome 11 (ref. 38). To establish the relationship between these dwarf mice and *pit-1*, the chromosome location of the *pit-1* gene was mapped by somatic cell genetics. The *pit-1* complementary DNA probe hybridized with two *Eco*RI-digested mouse DNA fragments (4.6 kilobases (kb) and 3.2 kb) and three *Eco*RI-digested hamster DNA fragments (3.6 kb, 2.4 kb and 2 kb) (Fig. 1a). Among the sixteen hybrid cell lines, all showed characteristic hamster hybridization, and a subset of these cell lines showed mouse-specific hybridization. Figure 1b compares the mouse chromosome content of these hybrid cell lines and their hybridization with the *pit-1* probe. Mouse chromosome 16 was present in all the cell lines that showed mouse-specific hybridization, and was absent in those that did not. None of the other mouse chromosomes met this criterion, permitting assignment of the *pit-1* gene to mouse chromosome 16. Because the *dw* mutation was previously mapped to mouse chromosome 16 (ref. 35), this colocalization suggested that the *pit-1* gene was a candidate for the *dw* locus.

A *pit-1* gene RFLP is present in *dw*^J

To determine whether the *pit-1* gene has undergone a gross structural change, we analysed restriction fragment length polymorphisms (RFLP) of the *pit-1* gene in both alleles of the *dw*

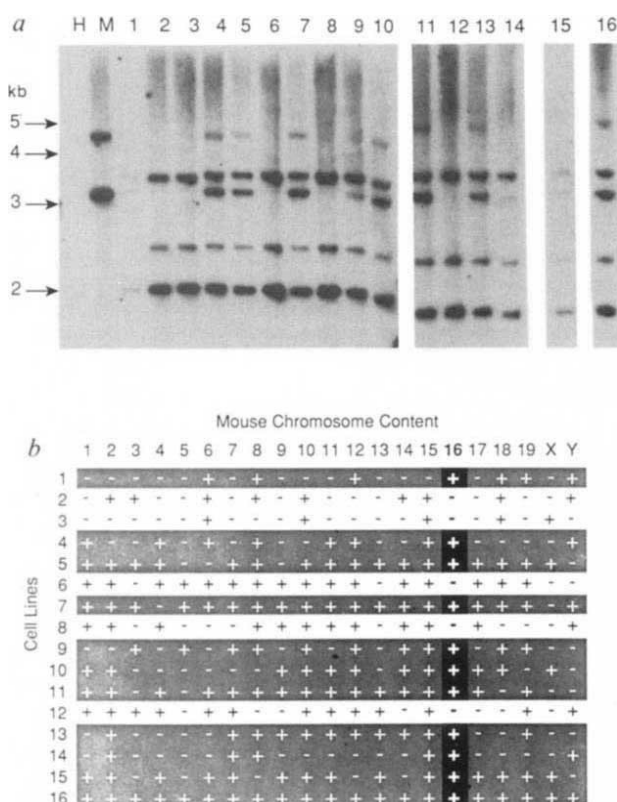


FIG. 1 Chromosome localization of *pit-1* gene. a, DNA blot analysis⁴⁵ of the mouse *pit-1* gene in mouse-hamster hybrid cell lines. H, hamster; M, mouse; each number represents a mouse-hamster hybrid cell line. All lines had a hamster restriction pattern (3.4 kb, 2.4 kb and 2 kb) and only some of the lines had mouse-specific hybridization (4.6 kb and 3.2 kb). b, Correlation of the mouse-specific hybridization and the mouse chromosome content. '+' indicates that a specific mouse chromosome is contained in that cell line; '-' indicates the absence of the particular mouse chromosome. The grey rows correspond to the cell lines showing mouse-specific hybridization on DNA blot analysis. Mouse chromosome 16 (bold) was present in all the cell lines showing a mouse-specific *pit-1* restriction pattern and was absent from all the cell lines that did not show mouse-specific hybridization.

METHODS. Approximately 10 µg DNA prepared from each of the sixteen different mouse/hamster hybrid cell lines was digested with *Eco*RI, electrophoresed in a 0.8% agarose gel, and transferred to nitrocellulose membranes. An *Eco*RI fragment of rat *pit-1* cDNA that contained most of the N-terminal portion of *pit-1* plus half of the sequence encoding the POU-specific domain¹⁷ was labelled by random priming to a specific activity $>10^9$ c.p.m. µg⁻¹. The filters were hybridized to this probe overnight at 42 °C in 50% formamide/10% dextran sulphate/6 × SSC, and then washed at 60 °C in 0.1 × SSC. The weak hybridization signal in some of the lanes was due to low quantities of DNA. The variation in the relative intensities of mouse versus hamster hybridization among cell lines is due to different percentages of cells within the cell line that contain the mouse chromosome (data not shown).

locus. The *pit-1* gene of Snell dwarf (*dw*) and dwarf Jackson (*dw^J*) was compared with that of corresponding wild-type strains. In Snell dwarf genomic DNA, no variations in restriction patterns of the *pit-1* gene between *dw* and wild-type strain were observed with any of the five restriction enzymes shown in Fig. 2a or three other restriction enzymes (*KpnI*, *SstI* and *XhoI*; data not shown). RFLPs were observed for dwarf Jackson in several restriction enzyme digestions, with patterns differing between *dw^J* and its wild-type strain (Fig. 2b). Because the *dw^J* allele arose on an inbred genetic background (C3H/HeJ), it was unlikely that the RFLPs result from variations among individual mice within the background strain. The variability of *pit-1* genomic structure among several inbred mouse strains (A/J, AKR/J, C57BL/6J, Balb/cJ, CBA/J, DBA/2J, C3H/HeJ and SJL/J) was also examined by *EcoRI* digestion; all of them had the same restriction patterns (data not shown). The different restriction patterns displayed by *dw^J* and its wild-type strain indicate that the structure of the *pit-1* gene itself, or a closely linked gene, is altered in *dw^J*.

The *pit-1* gene is disrupted in *dw^J*

To characterize further the mutation in dwarf Jackson, the mouse *pit-1* gene was isolated from a genomic library. Restriction mapping of eight positive clones indicated that the complete *pit-1* coding region was represented in two overlapping clones 61 and 32 (Fig. 3a). The mapped *EcoRI* sites and the genomic structure are presented in Fig. 3a. The six exons, labelled I–VI, encoded amino acids 1–47, 48–72, 73–147, 148–201, 195–221 and 222–291 of mouse Pit-1, respectively (Fig. 4). To map the dwarf Jackson mutation further, *EcoRI*-digested mutant and

wild-type genomic DNA were hybridized with the five adjacent *pit-1* genomic *EcoRI* fragments depicted in Fig. 3a (probe a contained only half of an *EcoRI* fragment ending at the 5' end of clone 32). Four of the five probes gave the expected restriction pattern with both normal and dwarf genomic DNA, whereas probe c hybridized to a single 3.2-kb fragment in normal DNA and two fragments (4.6 and 2.4 kb) in dwarf Jackson DNA. These data are consistent with a mutational event that resulted in either an inversion or an insertion of a DNA segment of >4 kb in the *pit-1* gene. On the basis of polymerase chain reaction (PCR) analysis, most of exon III is intact, but the exact break point has not been established. Regardless of which type of rearrangement event has occurred, the results clearly demonstrate that there is an disturbance in the *pit-1* gene in dwarf Jackson, suggesting that mutation of the *pit-1* gene accounts for the dwarf phenotype.

Point mutation in *pit-1* homeobox of *dw* mice.

Because the Snell dwarf did not reveal any altered RFLP bands (Fig. 2), we reasoned that the Snell dwarf mutation was likely to result from a point mutation in the gene. To test this hypothesis, *pit-1* cDNAs were cloned by PCR from normal mouse and Snell dwarf pituitary glands. To clone the normal mouse cDNA, two oligonucleotides corresponding to rat *pit-1* cDNA sequences were used; the first oligonucleotide corresponded to 5' sense strand noncoding sequences, with the 3' boundary of the oligonucleotide at the initiator methionine codon ATG; the second oligonucleotide corresponded to 3' antisense noncoding sequences with its 3' end encompassing the stop codon TAA. The high degree of similarity between rat and mouse sequences

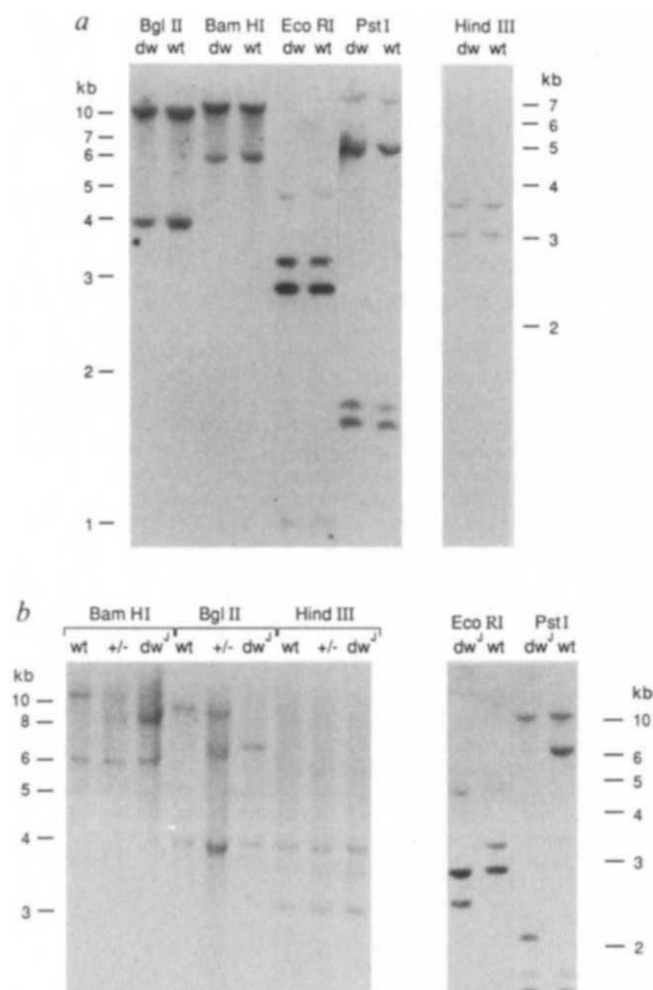


FIG. 2 Restriction analysis of Snell and Jackson *pit-1* genes. a, DNA blot analysis of Snell dwarf and wild-type DNA. Abbreviations: *dw*, homozygous Snell dwarf; *wt*, wild-type strain. Restriction patterns observed in *dw* and its wild-type siblings were similar with all five restriction enzymes. b, DNA blot analysis of dwarf Jackson and wild-type DNA. Abbreviations: *dw^J*, homozygous dwarf Jackson; *wt*, the wild-type strain (C3H/HeJ); +/- heterozygotes. *Bam*HI, *Bgl*III, *Eco*RI and *Pst* I digestion demonstrated structural changes associated with *pit-1* gene in dwarf Jackson mouse.

METHODS. Genomic DNA was prepared from the dwarf animals and their wild-type strains obtained from Jackson Laboratories (Bar Harbor). The dwarf Jackson mutation arose and is maintained on an inbred background (C3H/HeJ); the Snell dwarf mutation arose in strain of black-silver mice and was maintained on a background strain designated DW/J (distinct from the mutant *dw^J*)^{26,35}. DNA blots were prepared as described in Fig. 1, and probed with a mouse *pit-1* cDNA fragment encompassing the coding region (Fig. 4, nucleotides 60–846), with the exception of those blots containing DNA digested with *Bam*HI, *Bgl*III, and *Hind*III in b, which were probed with a 1.6-kb rat cDNA fragment¹⁷.

FIG. 3 Mouse *pit-1* genomic structure and the disruption in dwarf Jackson mice. **a**, Schematic representation of mouse *pit-1* gene. Two overlapping mouse λ FIXII genomic clones (clone 61 and clone 32) are represented by two straight lines with arrows marking the mapped *Eco*RI restriction sites. All exon-intron boundaries were defined by genomic DNA sequencing⁴⁸. The *pit-1* coding portion between the two dashed lines is enlarged to illustrate the exon-intron structure (see Fig. 4 for detail). The coding and noncoding exons are illustrated by the filled and open boxes, respectively. The *Eco*RI fragments (**a-e**) that were used to examine *pit-1* gene disruption in dwarf Jackson mutants are depicted underneath the genomic structure in the corresponding positions. **b**, DNA blot analysis of *pit-1* gene of *dw*^J mutants. The DNA blots are arranged from left to right corresponding to the probes from 5' to 3'. Letters **a-e** correspond to the letters in **a**; *dw*^J, homozygote dwarf Jackson; wt, wild-type strain. Only probe **c** revealed a structural difference in which the 3.2-kb *Eco*RI fragment in the wild type is disrupted in *dw*^J, resulting in two different fragments, 5.4 kb and 2.4 kb. The estimated sizes of the fragments are placed next to the marks that indicate their positions. **METHODS.** Duplicate filter lifts of λ FIXII mouse (B6/CBA) genomic library (Stratagene) were hybridized with two sets of oligonucleotides, each containing an oligonucleotide corresponding to N-terminal and C-terminal sequences. Set 1: (sense, nucleotides 58-83) TCTGC-TGCC-TGCCT-CTGAG-AATGCA; (antisense, nucleotides 824-847) TAGAG-AACAG-GCTCT-GATTG-AGAC. Set 2: (antisense, nucleotides 118-141) TGTGG-ACATC-ACGTT-GGTGG-CGTG; (sense, nucleotides 711-750) TCGCA-GGAGA-TCATG-CGGAT-GGCTG-AAGAA. From 10⁶ clones screened, eight primary plaques were positive for both sets of oligonucleotides. A mouse cDNA fragment (nucleotides 27-876), encompassing all four oligonucleotides, was used for secondary screening. On tertiary screening, one plaque hybridized to the N-terminal

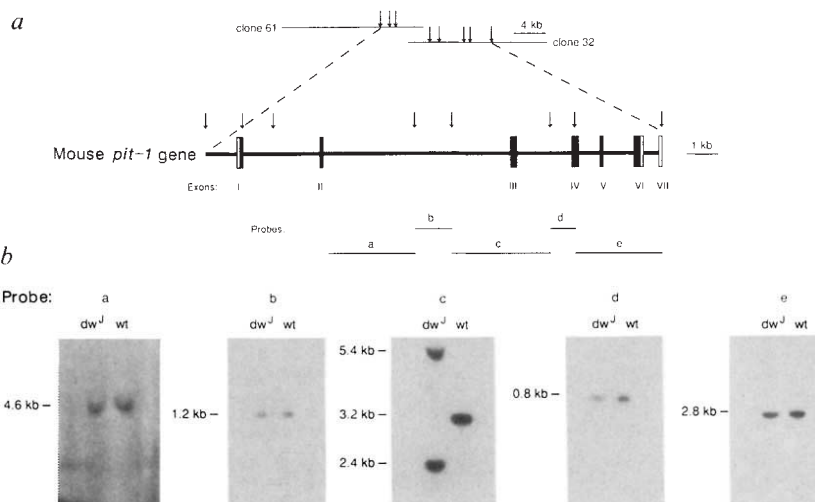


FIG. 4 Mouse *pit-1* cDNA sequence; exon-intron boundaries, and the point mutation in the Snell dwarf. Mouse *pit-1* cDNA sequence from ATG to TAA are shown, together with the protein sequence. The cDNA sequence was obtained from mouse pituitary mRNA by PCR, except for the sequence between nucleotides 1-58 and 847-876 that come from the mouse genomic sequence. The codon containing the point mutation found in Snell dwarf cDNA (G-to-T change) is marked by a black box as are the corresponding changes in amino-acid sequence from tryptophan to cysteine. The POU-specific domain is boxed and the POU homeodomain is underlined. The exact exon boundaries are marked by arrows and the numbering of the exons is the same as in Fig. 3.

METHODS. For the isolation of the initial normal mouse *pit-1* cDNA, total RNA, prepared from mouse pituitary by an acid guanidinium-phenol-chloroform method was reverse-transcribed into cDNA (5 μ g RNA template; oligo(dT) as a primer), and was then amplified by PCR⁴⁹ with degenerate oligonucleotides. Two rounds of amplification were necessary for isolation of enough *pit-1* fragment to clone; both rounds used the same 3' antisense oligonucleotide that encoded all possible codon combinations for the rat amino acids that correspond to residues 283 to the stop codon. For the first round of amplification, the 5' sense oligonucleotide encoded all possible codon combinations for the rat Pit-1 amino acids that correspond to residues 1-8; the second PCR included an oligonucleotide corresponding to residues 10-20. PCR-30 cycles (round 1) or 35 cycles (round 2) at 94 °C for 1 min, 50 °C (round 1) or 45 °C (round 2) for 2 min, and 72 °C for 3 min. Complementary DNA was generated as above from the total pituitary RNA of eight homozygote Snell dwarf mice (pituitary glands were a gift of Dr W. Beamer, Jackson Laboratory). The first round of PCR used two oligonucleotides terminating at the ATG start codon (sense, GTTCTCTACTCTCTTGTGGGAATG) or the TAA stop codon (antisense, GTACCGATGGTGTCCGTTTCAAGAT). One round of PCR amplification was sufficient to isolate enough fragment to clone from a small segment (<20%) of a normal mouse pituitary, but another round of PCR was necessary to isolate sufficient fragment from eight Snell dwarf pituitaries. The second round of PCR used oligonucleotides that lie within and slightly overlap those used in the first round of amplification (sense, GAATGAGTTGCCAACCTTTACCT; antisense, AGAATAGACGTGAGT-TCTACGAGG). PCR=35 cycles (round 1) or 20 cycles (round 2) at 94 °C for

oligonucleotide probe, and the other seven hybridized only to a C-terminal oligonucleotide probe. Phage DNAs were initially analysed by partial and complete restriction digestions, combined with DNA blot analysis⁴⁷ probed by either *pit-1* cDNA fragments or phage T3 and T7 primers that corresponded to sequences in the phage vector λ FIXII (data not shown). The overlap between the N-terminal clone and the C-terminal clones was first demonstrated using the end-labelled probe (Ref. 49; data not shown). The portion of genomic fragment encompassing the entire *pit-1* coding region was subcloned into plasmid pBKS II(-) (Stratagene). The exon-intron boundaries were determined by sequencing and the distances between exons were estimated by restriction analysis and confirmed by PCR. The Southern blots were carried out as described in Fig. 1, and the genomic DNA was digested using *Eco*RI.

Mouse Pit-1 cDNA Sequence and the Point Mutation in Snell Dwarf

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ATG AGT TGC CAA TCT TTC ACC TCG GGT GAC AAT TTT ATA ACT CTG AAT TGT GAC GGT 57
Met Ser Cys Gln Ser Phe Thr Ser Ala Asp Thr Phe Ile Thr Leu Asn Ser Asp Ala 19

TGT GGT GGC CTG CCT CTG AGA ATG CAC CAC AAT GGC GGT GAG TGT CCG CCA GCG TCC 114
Ser Ala Ala Leu Pro Leu Arg Met His His Ser Ala Ala Cys Leu Pro Ala Ser 38

AAC CAC GGC ACC AAC GTG ATG TCC ACA GCG ACA GSA CTT CAT TAC TGT GCG CCG TCC 171
Asn His Ala Thr Asn Val Met Ser Thr Ala Thr Gly Leu His Tyr Ser Val Pro Ser 57

TGT CAT TAT GSA AAC CAG CCA TCC ACC TAT GSA GTG ATG GCA GGC AGT TTA ACC CCG 213
Gly Thr Gly Asn Gln Pro Ser Thr Tyr Gly Val Met Ala Gly Ser Leu Thr Pro 76

TGT CTT TAC AAG TTT CCA GAC CAC ACC CTG AAT CAT GSA TTT CCG CCG CTG CAC CAG 255
Cys Leu Tyr Lys Phe Pro Asp His Thr Leu Ser His Gly Phe Pro Pro Leu His Gln 95

CCT CTG CTG GCA GAG GAC CCG GCG GCG TGT GAT TTT AAG CAG GAA CTC AGG CCG AAA 342
Pro Leu Leu Ala Glu Asp Pro Ala Ala Ser Glu Phe Lys Gln Glu Leu Arg Arg Lys 114

AGC AAA TTG GTG GAA GAG CCA ATA GAC ATG GAC TCC CCA GAA ATC CCA GAA CCG GAG 359
Ser Lys Leu Val Glu Glu Pro Ile Asp Met Asp Ser Pro Glu Ile Arg Glu [Leu Glu] 123

CAG TTT GGC AAC GAG TTT AAA GTG AGA ATA AAT AAG TTA GSA TAC ACC CAG ACA AAG 416
Gln Phe Ala Asn Glu Phe Lys Val Arg Arg Ile Lys Leu Gly Tyr Thr Gln Thr Asn 152

GTG GGC GAA GCG CTG GGT GGT GTC CAC GCG TCA GAA TTC AGT AAG ACA ACC ATC TGT 513
Val Gly Glu Ala Leu Ala Ala Val His Gly Ser Glu Phe Ser Gln Thr Thr Ile Cys 171

CGA TTT GAA AAC TCG CAG CTC AGT TTT AAA AAT GGT TGT AAA CTT AAA GCA ATT TTA 570
Arg Phe Glu Asn Leu Gln Leu Ser Phe Lys Asn Ala Arg Lys Leu Lys Ala Ile Leu 190

TCC AAG TGG CTG GAG GAA GCT GAG CAG CAT GTC GGA GGT TTT TAC AAT GAG AAG GTG GGA 617
Ser Lys Trp Leu Glu Glu Ala Glu Gln Val Gly Ala Leu Lys Tyr Asn Glu Lys Val Gly 219

GCA AAG GAA AGG AAG AGG AAA CCG AGG ACA ACC ATC AGT GTA GCT GGT AAG GAT GGT 634
Ala Asn Glu Arg Lys Arg Lys Arg Arg Thr Thr Ile Ser Val Ala Ala Lys Asp Ala 228

CTG GAG ACA CAC TTC GGG GAG CAC AGC AAG CCT TCC TCG CAG GAG ATC ATG CCG ATG 741
Leu Glu Arg His Phe Gly Glu His Ser Lys Pro Ser Ser Gln Glu Ile Met Arg Met 247

WT... GCT GAA GAA CTT AAT CTC GAG AAA GAA GTA GTA CGA GTC ACC TTC TGC AAC CGA AGG 738
dw... Ala Glu Glu Leu Asn Leu Glu Lys Glu Val Val Arg Val Trp Phe Cys Asn Arg Arg 266
dw... CAG AAG GAA AAA CGA GTG AAA ACA AGT CTC AAT CAG AGC CTG TTC CTT ATT TCC AAG 855
Gln Arg Glu Lys Arg Val Lys Thr Ser Leu Asn Gln Ser Leu Phe Ser Ile Ser Lys 285

GAA CAT CTA GAG TGC AGA TAA
Glu His Leu Glu Cys Arg *
876
291

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1 min, 50 °C for 2 min, and 72 °C for 3 min. The reaction mixtures were separated on an agarose gel and the DNA fragment of expected size was subcloned and sequenced by the dideoxy chain termination method⁴⁸. The characterization of exon boundaries has been described in Fig. 3.

easily permitted the isolation of *pit-1* from normal mouse pituitaries (Fig. 4). But the oligonucleotides did not amplify a discernable band from Snell dwarf pituitary cDNA. A second round of amplification, using two more internal oligonucleotides, was necessary to isolate Snell dwarf *pit-1* cDNA. Two independent subclones of the PCR product were sequenced, and comparison of the mutant and wild-type sequences revealed a G-to-T alteration in the Snell dwarf *pit-1* cDNA that would convert the tryptophan residue in the POU-homeodomain (Trp 261) to a cysteine residue (Fig. 4). To exclude the possibility of PCR artefacts, oligonucleotides encompassing the mutation were designed on the basis of genomic sequences in exon VI, and one of these oligonucleotides corresponded to sequences that fell outside the cDNA clone; this strategy excluded the possibility of reamplifying any previously isolated cDNA products, thereby avoiding any artefacts due to contamination. PCR products from each of the normal genomic DNA gave the wild-type sequence, but each of the four Snell dwarf PCR preparations showed the G→T mutation of the cDNA clones (Fig. 5a, b). The mutated tryptophan residue is conserved in organisms from mammals to yeast, and exists in all classes of the POU- and homeodomain proteins (reviewed in refs 2, 39, 40). The importance of this tryptophan residue in the function of Pit-1 is illustrated by the fact that this mutation abolishes binding to a high affinity *pit-1* site (Prl-1P; data not shown), and mutations of this region in Oct-1 (tryptophan, phenylalanine, cysteine residues) also abolish DNA binding⁴¹.

Expression of *pit-1* in *dw*, *dw¹* and Ames dwarfs

Having characterized mutations of the *pit-1* gene in *dw¹* and Snell dwarfs, we determined using immunohistochemistry and hybridization histochemistry whether the expression of *pit-1* messenger RNA and protein is normal in these mutants. In contrast to the wild-type controls, neither mRNA nor protein were detected in either of the dwarf mice (Fig. 6). The failure to detect *pit-1* mRNA or protein in the dwarf Jackson pituitary

indicates that *pit-1* expression was impaired or abolished as a consequence of the gross structural alterations in the gene. The finding that *pit-1* transcripts in Snell dwarf mouse could be amplified only by the PCR, together with the results of the *in situ* hybridization and immunohistochemical studies, suggests that there is a low level of *pit-1* gene expression in the Snell dwarf.

Because an understanding of the effect of the Ames dwarf mutation on the expression of *pit-1* would help to elucidate the mechanisms that lead to similar phenotypes for these two dwarf loci, similar experiments were conducted on the Ames dwarf pituitary with virtually identical results (Fig. 6). The lack of detectable *pit-1* gene expression in the phenotypically similar Ames dwarf indicated that the Ames mutation is epistatic to the expression of *pit-1*. Thus, the Ames dwarf locus might be involved in the regulation of *pit-1*, or perhaps, in conjunction with *pit-1*, for the specification and/or maintenance of the three specific pituitary cell types affected by the mutation.

Discussion

The characterization of tissue-specific gene activation in mammalian organ systems has provided a wealth of information about the phenotypes of mature cells, but clear insight into the role of these factors during developments has been hampered by the lack of a genetic animal model system. Here we have shown that both alleles of the *dw* locus result from mutations in the gene encoding the pituitary-specific transcription factor, Pit-1. In Snell dwarfs, a point mutation in the *pit-1* gene alters the functionally critical tryptophan residue that is invariant throughout eukaryotic evolution in the POU- and homeodomain transcription factors. These dwarf genetic models therefore provide the initial genetic evidence for the role of a well characterized transcription factor during mammalian organogenesis.

Because mutations in the *pit-1* gene result in the loss of three pituitary cell types in the *dw* mutants, *pit-1* must activate a part of the pituitary developmental program. The absence of the

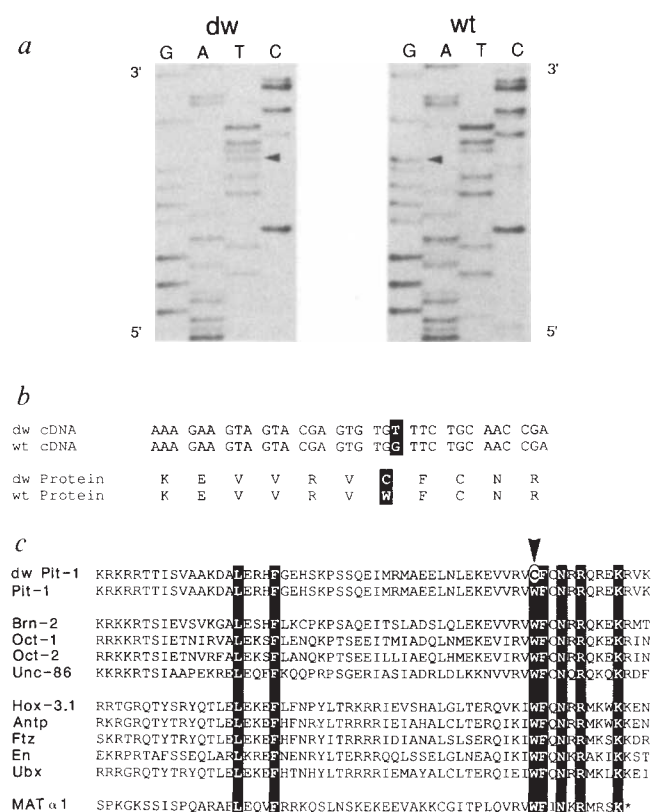
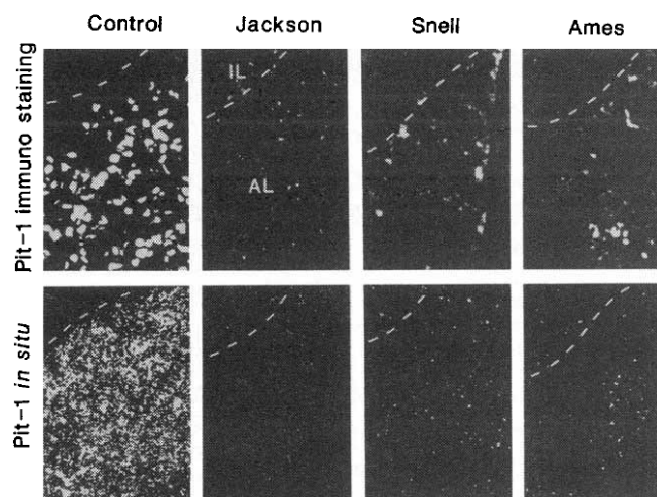


FIG. 5 Confirmation of the point mutation of the highly conserved Trp 261. **a**, Sequence gel of Snell dwarf genomic fragment across the point mutation. The arrows mark the nucleotide change revealed on the sequence gel. The orientation of the sequence gel is indicated, as well as the nucleotides in each lane. Abbreviations: *dw*, homozygote Snell dwarf; *wt*, wild-type strain. **b**, Sequence represented in the gel of **a** and the corresponding protein sequence. The mutation, which causes a G-to-T change is highlighted by bold white letters in the black box, as is the resulting change in the amino acid Trp to Cys. **c**, Evolutionary conservation of Trp 261. The amino-acid sequences of different classes of POU homeodomains and classical homeodomains were aligned with the mutated Pit-1 POU homeodomain. The Trp 261 which is mutated to Cys (bold in the circle under the arrow) in Snell (*dw* Pit-1) is one of the seven (white bold letters in the black boxes) amino acids that are invariantly conserved in all the POU-domain and homeodomain proteins. Abbreviations: *dw* Pit-1, Snell dwarf mouse Pit-1; Pit-1, mouse Pit-1; Brn-2 (ref. 39); Oct-1; Oct-2; Unc-86 (reviewed in ref. 22); Hox 3.1, mouse homeobox gene *Hox* 3.1 product, Antp, *Antennapedia* gene product; Ftz, *fushi tarazu* gene product; En, *engrailed* gene product; Ubx, *Ultrabithorax* gene product (see ref. 2); MAT α1, yeast mating type regulatory gene product⁴⁰. **METHODS**. Two oligonucleotides corresponding to sequences ~150 base pairs, apart, sense oligonucleotide (nucleotides 711–750) and the antisense oligonucleotide CACACATGGCTATCACAGCCAGC, designed on the basis of mouse genomic sequence outside the TAA at which the mouse and rat sequences diverge were used in a PCR of the genomic DNA from four Snell dwarf mutants and two wild-type animals. The amplified fragments were subcloned and sequenced as shown.

FIG. 6 Pit-1 immunostaining (top) and *in situ* hybridization (bottom) of tissue sections from 3–4 week old control and dwarf mouse pituitaries. In control mice, detectable immunostaining for Pit-1 protein was confined to cell nuclei in the anterior lobe (AL), but was absent in sections from Jackson, Snell and Ames dwarf mice. Small bright structures in the sections from mutant mice were erythrocytes, which autofluoresced orange, and were thus easy to distinguish from the specific Pit-1 immunofluorescence (green due to the fluorescein-conjugated secondary antiserum) when viewed under epifluorescent illumination. *In situ* hybridization specific for *pit-1* mRNA (silver grains appear white in these darkfield photomicrographs) were confined to the AL control mice. In sections from mutant mice, silver grain counts show that background labelling was uniformly distributed over the AL and intermediate lobe (IL); boundaries between IL and AL are indicated by dashed white lines and were determined by viewing a fluorescent counterstain (bisbenzidine, not visible with the illumination used for photography.)

METHODS. The immunohistochemical and hybridization histochemical methods and reagents were as those described in detail elsewhere¹⁰. Fresh pituitaries were dissected out and fixed immediately in 4% paraformaldehyde in 0.1 M borate buffer (pH 9.5), plus 10% sucrose for 12 h. Then, 5 μ m-thick, frozen sections were cut on a cryostat. A 450-nucleotide [³⁵S]-complementary RNA probe directed towards the 5' end of the *Pit-1* mRNA⁴⁰ was used for *in situ* hybridization at a concentration of 5×10^6 c.p.m. ml⁻¹.



three cell types corresponds to the cells in which *pit-1* expression is observed in the normal pituitary gland⁷. The analyses of *dw* mutants prove that *pit-1* is required for the specification of somatotroph and lactotroph phenotypes, which includes the activation of growth hormone and prolactin gene expression. These data argue against the suggestion that a subset of lactotrophs are derived by way of a Pit-1 independent pathway⁴², and that the role of *pit-1* is restricted to the specification of somatotroph phenotype^{14,21}. The evidence provided by the genetic dwarf locus clearly demonstrates a broader role of *pit-1* in pituitary organogenesis.

Although the specific function of *pit-1* in thyrotrophs is not so far clear, the disappearance of TSH cells in *pit-1*-defective dwarf animals implies an important role for *pit-1* in the specification and/or maintenance of thyrotroph phenotype. The appearance of thyrotrophs (β TSH) before the detectable expression of the *pit-1* gene may indicate that *pit-1* is required for cell survival and proliferation of TSH-producing cells, possibly by the regulation of cell machinery that responds to trophic factors. But it is possible that *pit-1* expression occurs at levels below the limits of detection before β TSH expression, and is required for the specification of the thyrotroph cell phenotype.

Homeodomain proteins in *Drosophila*, *C. elegans* and mouse

have important developmental roles^{1–3,43}. Mutations of the POU-domain protein encoded by *unc-86* in *C. elegans* affect several cell lineages throughout the body, or block the progression from immature to mature cell phenotypes^{4,25}. In the pituitary, the progression from immature cells to mature somatotrophs and lactotrophs could involve a pool of pre-somatotroph stem cells^{44–46}. The observation of putative immature cells with histological characteristics of both somatotrophs and lactotrophs in Snell mice^{33,34} suggests a role for *pit-1* in the developmental progression of stem cells to a mature pituitary cell type.

The hypoplastic nature of the dwarf pituitary indicates that cell proliferation and/or survival are also a component of the programme specified by *pit-1*. These data are consistent with a role of *pit-1* in the regulation of other genes—such as those encoding growth factor receptors or receptors for hypothalamic trophic factors—that are required in the proliferation and developmental progression of stem cells to a mature pituitary cell type. By permitting the characterization of gene products absent in hypoplastic dwarf pituitaries, the genetic dwarf model system will allow the full characterization of the developmental programme specified by *pit-1* during pituitary organogenesis. □

Received 29 May; accepted 15 August 1990.

- Gehring, W. J. *Science* **236**, 1245–1252 (1987).
- Scott, M. P., Tamkun, J. W. & Hartzell, G. W. *Biochim. biophys. Acta* **989**, 25–48 (1989).
- Ingham, P. W. *Nature* **335**, 25–34 (1988).
- Chalfie, M., Horvitz, H. R. & Sulston, J. E. *Cell* **24**, 59–69 (1981).
- Schwind, J. *Am. J. Anat.* **41**, 295–319 (1928).
- Watanabe, Y. G. & Daikoku, S. *Dev. Biol.* **68**, 557–567 (1979).
- Simmons, D. M. *et al. Genes Dev.* **4**, 695–711 (1990).
- Neelson, C. *et al. Nature* **322**, 557–562 (1986).
- Nelson, C., Albert, V. R., Elsholtz, H. P., Lu, L. E.-W. & Rosenfeld, M. G. *Science* **239**, 1400–1405 (1988).
- Cao, Z., Barron, E. A., Carrillo, A. J. & Sharp, Z. D. *Molec. cell. Biol.* **7**, 3402–3408 (1987).
- Lufkin, T. & Bancroft, C. *Science* **237**, 283–286 (1987).
- Schuster, W. A., Treacy, M. N. & Martin, F. *EMBO J.* **7**, 1721–1727 (1988).
- West, B. L. *et al. Molec. cell. Biol.* **7**, 1193–1197 (1987).
- Bodner, M., Castrillo, J.-L., Theil, L. E., Deerinck, T., Ellisman, M. & Karin, M. *Cell* **55**, 505–518 (1988).
- Crenshaw, III, E. B., Kalia, K., Simmons, D. M., Swanson, L. W. & Rosenfeld, M. G. *Genes Dev.* **3**, 959–972 (1989).
- Ye, Z. S. & Samuels, H. H. *J. Biol. Chem.* **262**, 6313–6317 (1987).
- Ingraham, H. A. *et al. Cell* **55**, 519–529 (1988).
- Mangalam, H. J. *et al. Genes Dev.* **3**, 946–958 (1989).
- Larkin, S., Tait, S., Treacy, M. N. & Martin, F. *Eur. J. Biochem.* (in the press).
- Fox, S. R. *et al. Molec. Endocr.* **4**, 1069–1080 (1990).
- Castrillo, J.-L., Bodner, M. & Karin, M. *Science* **243**, 814–817 (1989).
- Herr, W. *et al. Genes Dev.* **2**, 1513–1516 (1988).
- Kemler, I. & Schaffner, W. *FASEB J.* **4**, 1444–1449 (1990).
- Ingraham, H. A. *et al. A. Rev. Physiol.* **52**, 773–791 (1990).
- Finney, M., Ruvkun, G. & Horvitz, H. R. *Cell* **55**, 757–769 (1988).
- Snell, G. D. *Proc. natn. Acad. Sci. U.S.A.* **15**, 733–734 (1929).
- van Buul-Offer, S. *Acta endocr. Suppl.* **258**, 7–47 (1983).

- Smith, P. E. & MacDowell, E. D. *Anat. Rec.* **46**, 249 (1930).
- Smith, P. E. & MacDowell, E. D. *Anat. Rec.* **50**, 85 (1931).
- Carsner, R. K. & Rennels, E. G. *Science* **131**, 829 (1960).
- Roux, M., Bartke, A., Dumont, F. & Dubois, M. P. *Cell Tissue Res.* **223**, 415–420 (1982).
- Yashiro, T., Arai, M., Miyashita, E., Yamashita, K. & Suzuki, T. *Cell Tissue Res.* **251**, 249–255 (1988).
- Wilson, D. B. & Wyatt, D. P. *Anat. Rec.* **215**, 282–287 (1986).
- Wilson, D. B. & Wyatt, D. P. *Anat. Embryol.* **174**, 277–282 (1986).
- Eicher, E. M. & Beamer, W. G. *J. Hered.* **71**, 187–190 (1980).
- Schaible, R. & Gowen, J. W. *Genetics* **56**, 896 (1961).
- Cheng, T. C. *et al. Endocrinology* **113**, 1669–1678 (1983).
- Mouse News Letter* **66**, 4–35 (1982).
- He, X. *et al. Nature* **340**, 35–42 (1989).
- Herskowitz, I. *Nature* **342**, 749–757 (1989).
- Sturm, R. A. & Herr, W. *Nature* **336**, 601–604 (1988).
- Dollé, P. *et al. Cell* **60**, 809–820 (1990).
- Balling, R., Deutsch, U. & Gruss, P. *Cell* **55**, 531–535 (1988).
- Heffler, J. P., Bookfor, F. R. & Frawley, L. L. *Endocrinology* **117**, 187–195 (1985).
- Behringer, R. R., Mathews, L. S., Palmiter, R. D. & Brinster, R. L. *Genes Dev.* **2**, 453–461 (1988).
- Borrelli, H. R., Arias, C., Sawchenko, P. E. & Evans, R. *Nature* **339**, 267–275 (1989).
- Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).
- Sanger, F., Nicklen, S. & Coulson, A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
- Wahl, G. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **84**, 2160–2164 (1987).
- Saiki, R. K. *et al. Science* **239**, 487–449.

ACKNOWLEDGEMENTS. The contributions of S. Li and E. B. Crenshaw to this manuscript were equivalent, and both authors should be considered primary author. We thank K. Kalia, S. R. Dillon, H. Ingraham and M. Treacy for contributing to this study; Xi He, D. Drolet, K. Scully and J. Voss for discussions and suggestions; W. Beamer for helpful discussions and for providing Snell mice; R. Sparkes for providing mouse-hamster hybrid DNA; S. Camper for Ames dwarf pituitary glands; and S. Inglis for help in preparing the manuscript. This study was supported by the NIMDDK, E.B.C. is supported by the NIH. M.G.R. is an investigator with the Howard Hughes Medical Institute.

Discovery of an X-ray burst from X2127+11 in the globular cluster M15

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THE nature and evolution of binary X-ray sources in globular clusters are poorly understood. Among the ~ 10 bright X-ray sources in globular clusters, X2127+11 in M15 is very important because it is the only one for which the optical counterpart, AC211, and orbital period, 8.5 h, are both known¹. We have detected a type I X-ray burst from X2127+11, which was the only bright X-ray source in a globular cluster from which no burst had been detected. The burst was of long duration (≥ 150 s) and had a precursor separated from the main peak by ~ 6 s. This means that the burst was very energetic and that a large photospheric expansion occurred. The observed burst peak luminosity, well above 10^{38} erg s⁻¹, is hard to reconcile with the standard idea that X2127+11 is screened from direct view by an accretion disk. This latter view arises from consideration of the low ratio of X-ray to optical luminosity, which suggests that only a small fraction of X-rays is visible through scattering by an accretion-disk corona around the source²⁻⁴.

We observed X2127+11 in the globular cluster M15 with the large-area proportional counters⁵ (LAC) on the Ginga satellite⁶. The observations were carried out on 20–25 October 1988, and the net observation time (excluding the Earth occultation of the

source and high-background portions such as south Atlantic anomaly passages) was $\sim 1.0 \times 10^5$ s. During the observations the average X-ray flux of X2127+11 was 5×10^{-10} erg cm⁻² s⁻¹ (1–28 keV), and we observed erratic intensity variations of a factor of about three in addition to a clear 8.5-h orbital modulation⁷. We detected an energetic X-ray burst from X2127+11 on 20 October 17:34:50 UT (onset time). We could not observe the end of the burst because the Earth occulted X2127+11.

Although the LAC has a relatively wide field of view ($1^\circ \times 2^\circ$ full width at half maximum), it is clear that the burst came from X2127+11 because no other known X-ray source was in the field of view and because there was a significant decrease in the persistent X-ray flux between the precursor and the main peak of the burst (Fig. 2). The burst must therefore have come from the source of the persistent emission, X2127+11.

There are two kinds of X-ray bursts^{8,9}. Type I bursts are observed from all X-ray burst sources, whereas type II bursts have so far only been observed in the Rapid Burster. Type I bursts are characterized by a temperature decrease and an emission region of approximately constant size during the burst decay. Type II bursts, on the other hand, are characterized by an approximately constant temperature and a decrease in the size of the emission region during the burst decay. It is believed that type I bursts result from the runaway of thermonuclear burning on the surface of a neutron star, whereas type II bursts result from instability of the mass accretion onto the neutron star. In Fig. 1, we show the count rate plotted against time for the burst from X2127+11 in four energy bands. This burst is extremely energetic and the large photospheric expansion distinguishes it from the usual type I bursts. But there was a temperature decrease in the decay of the burst (after 17:36:30) indicated by the difference in the decay timescale between the higher and the lower energy bands, and we therefore conclude that the burst was a type I burst.

The burst had a precursor that preceded the main peak by ~ 6 s. Such bursts with precursors have been observed from several sources^{10,11}. They are extremely energetic, and the precursor has been explained by photospheric expansion as a

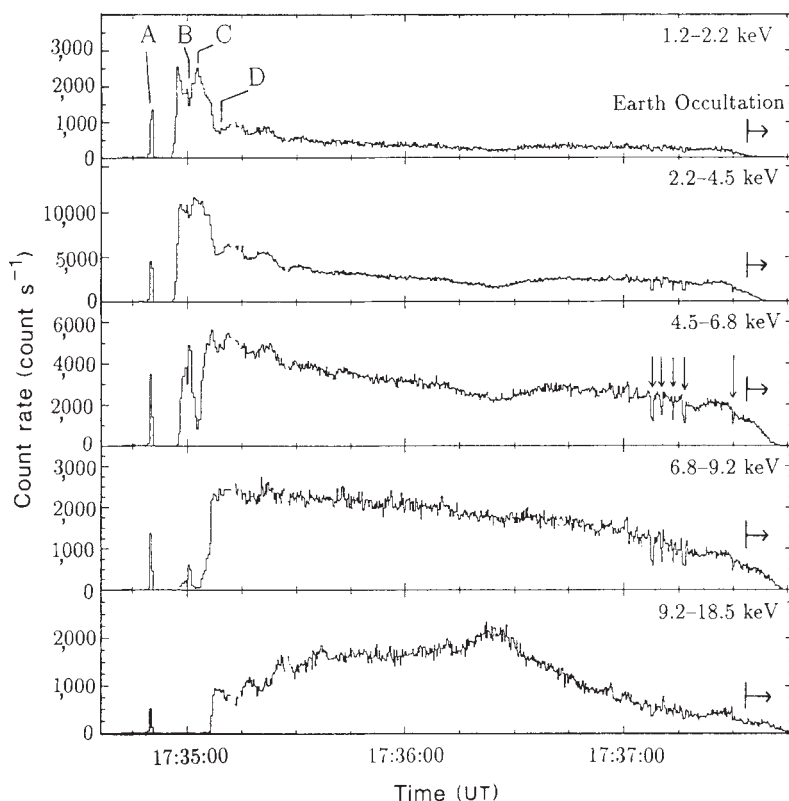
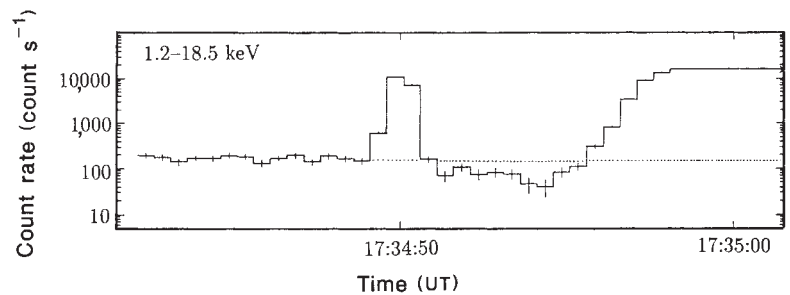


FIG. 1 Plot of the count rate against time of the type I X-ray burst from X2127+11. The observation was made in 48 energy channels (0.5-s time resolution) until just after the peak of the burst and then switched to 12-energy-channel mode (7.8-ms time resolution); the latter data are re-binned to 0.25-s time bin in the figure. Non-X-ray and diffuse-X-ray background have been subtracted, and the data corrected for aspect. The rapid decrease of the count rate after 17:37:35 is an artefact owing to Earth occultation of the source. Five dips are indicated by arrows near the end of the burst. Marks A–D indicate times of the energy spectra shown in Fig. 3a–d, respectively.

FIG. 2 Count rate at the onset of the burst. Data are plotted on a logarithmic scale to show the change in the persistent emission between the precursor and the main part of the burst. The persistent emission level before the burst occurrence is indicated by the dotted line. Data have been corrected as in Fig. 1.



consequence of excessive radiation pressure^{12,13}. The precursor corresponds to the burst onset. The luminosity quickly reaches the Eddington limit, and the photosphere of the neutron star expands very rapidly. This reduces its temperature and the bulk of the emission is shifted to lower photon energies outside the X-ray band, forming a precursor. After a few seconds, the excess radiation pressure diminishes and the photosphere begins to contract. As a result, the temperature rises and X-ray emission resumes from the low-energy end.

The burst profile has several notable features: (1) significant X-ray flux between the precursor and the main part of the burst; (2) oscillations of the X-ray flux as the photosphere contracts; (3) five dips towards the end of the burst. The X-ray flux between the precursor and the main part of the burst is shown in Fig. 2. This X-ray flux was about half of the persistent emission before the burst occurred. Although the count statistics during this period are poor, no qualitative change in the spectrum is evident.

During this period, the cooled photosphere of the neutron star must have been large enough (more than several hundreds kilometres) to cover the inner part of the accretion disk. As the photosphere is too cool to emit X-rays and the X-ray emission from the inner part of the accretion disk is screened by the photosphere, the origin of the remaining X-rays is unclear.

The oscillations of the X-ray flux have a period of ~ 10 s and can be seen for several cycles in the course of contraction of the photosphere (Fig. 1). There is a phase reversal between the soft- and the hard-energy bands. Similar oscillations showing an energy-dependent phase reversal have been observed during a big burst from X1608-52 (ref. 14). Those oscillations were interpreted as the radial oscillations of an envelope supported by radiation pressure near the Eddington limit¹⁵. Although the period of oscillation of X2127+11 (~ 10 s) is much longer than that of X1608-52 (0.65 s), there may be a common mechanism.

The five dips (marked by arrows in Fig. 1) near the end of

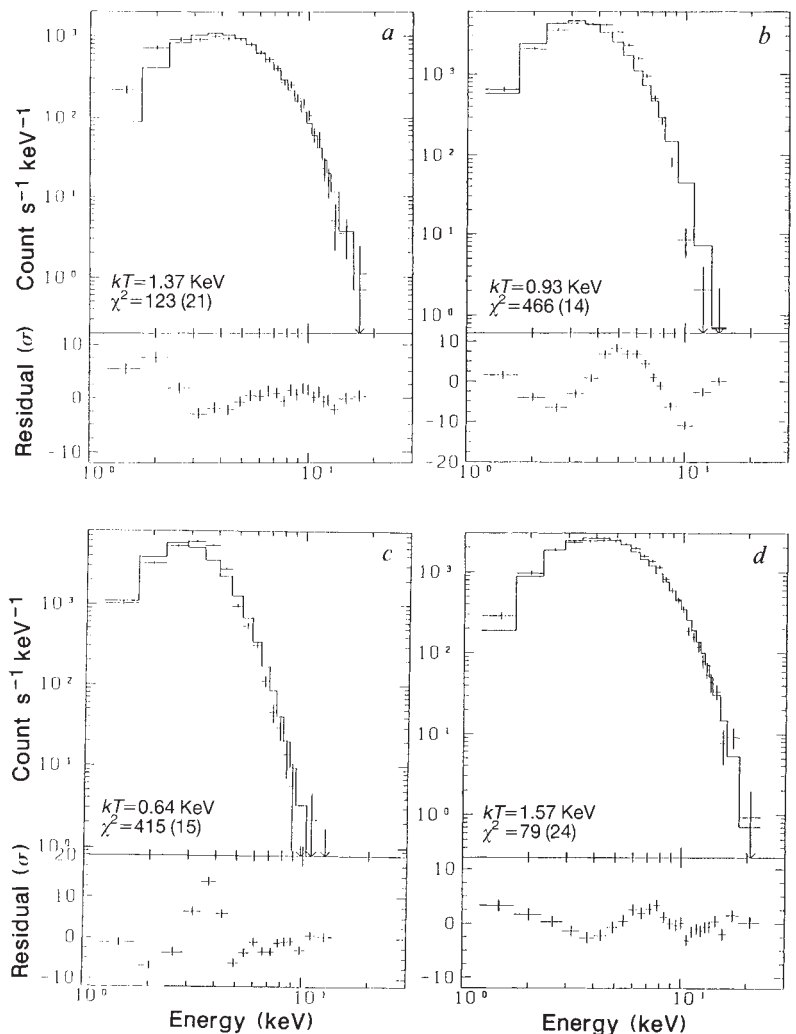


FIG. 3 Four representative energy spectra of the burst during the photospheric expansion (*a-c*) and after contraction of the photosphere (*d*) (times indicated by A-D in Fig. 1). Non-X-ray and diffuse-X-ray background have been subtracted (see text). The best-fit blackbody is shown by histograms. The colour temperature of the blackbody and χ^2 of the fit (degree of freedom in parentheses) are indicated. The energy spectra deviate from the blackbody spectrum during the photospheric expansion (*a-c*), but the energy spectrum in *d* is relatively well approximated by a blackbody.

the burst have a duration of about one second and are separated by about three seconds, except for the fifth one. Because the depth of the dips is almost independent of the X-ray energy, the dips, if caused by photoelectric absorption, must be due to clouds that are opaque to X-rays at least up to 10 keV. Furthermore, the size of the cloud is such that it occults only about half of the emission region. Such dips of short duration are unusual, and seem to be different from the transient dips observed from partial eclipsing binaries¹⁶.

The energy spectrum of type I bursts is generally well expressed by a blackbody spectrum with a colour temperature decreasing as the burst decays⁹. Our spectra exhibit significant deviations from blackbody spectra, however, particularly when the best-fit colour temperature is below 1.5 keV. Figure 3 shows four examples of observed spectra (at the times indicated in Fig. 1) together with best-fit blackbody spectra. We subtracted non-X-ray and diffuse-X-ray background from the data because the persistent emission level may have changed during the burst, but the burst was so energetic that the choice of the background had little effect on the spectra obtained. More than a simple hard-X-ray tail is required to explain the deviation of the spectra from a blackbody. By contrast, when the colour temperature is high ($kT > 1.5$ keV), the spectra are relatively well approximated by a blackbody. Such a deviation of the burst spectra from that of a blackbody has not been noted before and its origin is unknown.

The bolometric correction for the determination of the peak flux of the burst is somewhat uncertain because of this deviation from a blackbody spectrum. We therefore integrated the observed flux above 2 keV and corrected only for the energy-dependent detection efficiency, which gives $>90\%$ of the total flux when $kT > 1.5$ keV. Thus the luminosity just after the contraction of the photosphere is estimated to be $\sim 5 \times 10^{38}$ erg s⁻¹ for the well estimated distance to M15 of 10 kpc (ref. 17). This value is much larger than the Eddington limit for a neutron star of $M = 1.4 M_{\odot}$ and $R = 10$ km. The calculation of the radius of the photosphere and the effective temperature is not straightforward because of the deviation from a blackbody spectrum, general relativistic effects, the difference between effective temperature and colour temperature, and the possible anisotropy of the emission. A detailed discussion can be found in van Paradijs *et al.*¹⁸.

The type I burst from X2127+11 confirms that the compact object of this binary source is a neutron star. The X2127+11/AC211 system has relatively small ratio of the X-ray to optical luminosities, L_X/L_{opt} , compared to usual values for low-mass X-ray binaries^{2,3,19,20}. A plausible interpretation of the low L_X/L_{opt} value is that the central X-ray source is screened from direct view by the accretion disk (an inclination angle near 90°) and that only a small fraction of the X-rays is observable after scattering by some surrounding matter (such as the accretion disk corona²¹). Most of the models of the X2127+11/AC211 system have been based on this picture²⁻⁴, but the type I X-ray burst from X2127+11 is inconsistent with this. The observed peak luminosity of the burst, $\sim 5 \times 10^{38}$ erg s⁻¹, is in excess of the Eddington limit for a $1.4 M_{\odot}$ neutron star, which itself requires separate discussion¹⁸. The observed X-rays can hardly be a small fraction of the total burst emission, implying that the neutron star is directly visible. Consequently, the persistent luminosity, $\sim 6 \times 10^{36}$ erg s⁻¹, must be the unobscured source luminosity. This idea is consistent with the following observations, believed to be characteristic of a low-luminosity source^{22,23}: (1) X2127+11 produces an X-ray burst and the burst is of long duration (≥ 150 s); (2) the energy spectrum of the persistent emission is a power-law type¹⁸. The lack of any X-ray eclipses is also consistent with the idea that we are directly viewing the central X-ray source²⁴. This result indicates that a low L_X/L_{opt} does not necessarily mean that the source is screened from direct view. The optical luminosity, most of which is believed to come from reprocessing of X-rays, may be

enhanced owing to an extended accretion disk corona, low metallicity of M15 and the low inclination of the binary system²⁵. □

Received 13 June; accepted 14 August 1990.

- Charles, P. A. & Naylor, T. *Adv. Space Res.* **8**, 515–523 (1988).
- Charles, P. A., Jones, D. C. & Naylor, T. *Nature* **323**, 417–419 (1986).
- Naylor, T., Charles, P. A., Drew, J. E. & Hassall, B. J. M. *Mon. Not. R. astr. Soc.* **233**, 285–304 (1988).
- Bailyn, C. D., Garcia, M. R. & Grindlay, J. E. *Astrophys. J.* **344**, 786–790 (1989).
- Turner, M. J. L. *et al. Publ. astr. Soc. Japan* **41**, 345–372 (1989).
- Makino, F. *et al. Astr. Lett. Commun.* **25**, 223–233 (1987).
- Callanan, P. J., Naylor, T. & Charles, P. A. in *Accretion-Powered Compact Binaries* (ed. Mauche, C. W.) (Cambridge University Press, in the press).
- Hoffman, J. A., Marshall, H. L. & Lewin, W. H. G. *Nature* **271**, 630–633 (1978).
- Lewin, W. H. G. & Joss, P. C. *Space Sci. Rev.* **28**, 3–88 (1981).
- Hoffman, J. A. *et al. Astrophys. J.* **221**, L57–L62 (1978).
- Tawara, Y. *et al. Astrophys. J.* **276**, L41–L44 (1984).
- Lewin, W. H. G., Vacca, W. D. & Basinska, E. M. *Astrophys. J.* **277**, L57–L60 (1984).
- Tawara, Y., Hayakawa, S. & Kii, T. *Publ. astr. Soc. Japan* **36**, 845–853 (1984).
- Murakami, T., Inoue, H., Makishima, K. & Hoshi, R. *Publ. astr. Soc. Japan* **39**, 879–886 (1987).
- Shibazaki, N. & Ebisuzaki, T. *Publ. astr. Soc. Japan* **41**, 641–650 (1989).
- Mason, K. O. in *The Physics of Accretion onto Compact Objects* (eds Mason, K. O., Watson, M. G. & N. E. White) 29–57 (Springer-Verlag, New York, 1986).
- Fahlmann, G. G., Richer, H. B. & VandenBerg, D. A. *Astrophys. J. Suppl. Ser.* **58**, 225–254 (1985).
- Van Paradijs, J., Dotani, T., Tanaka, Y. & Tsuru, T. *Publ. astr. Soc. Japan* (in the press).
- Auriere, M., Maucherat, A., Cordoni, J.-P., Fort, B. & Picat, J. P. *Astr. Astrophys.* **158**, 158–160 (1986).
- Ilovaisky, S. A. *et al. Astrophys. J.* **179**, L1–L4 (1987).
- White, N. E. & Holt, S. S. *Astrophys. J.* **257**, 318–337 (1982).
- Van Paradijs, J., Penninx, W. & Lewin, W. H. G. *Mon. Not. R. astr. Soc.* **233**, 437–450 (1988).
- Mitsuda, K., Inoue, H., Nakamura, N. & Tanaka, Y. *Publ. astr. Soc. Japan* **41**, 97–111 (1989).
- Callanan, P. J., Fabian, A. C., Tennant, A. F., Redfern, R. M. & Shafer, R. A. *Mon. not. R. astr. Soc.* **224**, 781–789 (1987).
- Fabian, A. C., Guilbert, P. W. & Callanan, P. J. *Mon. Not. R. astr. Soc.* **225**, 29P–31P (1987).

ACKNOWLEDGEMENTS. We thank J. van Paradijs for a review.

Evidence from solar seismology against non-standard solar-core models

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GLOBAL oscillations of the Sun¹ have been used to test solar models², but modelling the oscillation frequencies to their measured accuracies of a few microhertz has proved difficult, mostly owing to ignorance of the structure of the Sun's outer layers³. The frequency separation between closely spaced modes in the acoustic spectrum is expected to depend more on core properties⁴, however, and thus to provide constraints on models of the solar core. Our observations combine data from a global network of observing stations, which reduces the masking effect of daily sidebands in the spectral analysis. Here we present precision measurements of fine structure and its variation with frequency. Our results agree with standard solar models^{5–7}, and seem to remove the need for significant mixing^{8,9} or weakly interacting massive particles (WIMPS)^{10,11} in the core, both of which have been advanced to explain the low measured flux of solar neutrinos^{12,13}. This suggests that the solar neutrino problem must be resolved within neutrino physics, not solar physics; neutrino oscillations and a finite neutrino mass form a possible explanation.

Only three probes of the deep interior of the Sun are available to us at present—a possible gravitational quadrupole moment, solar neutrinos and solar seismology. The first of these could be due to rapid rotation or intense magnetic fields¹⁴ and is a higher-order term of the gravitational potential, which could perturb the orbits of objects in the vicinity of the Sun, such as the planet Mercury or a future space probe. The excellent agreement between theoretical expectations of general relativity and observations of the rotation of the perihelion of Mercury and independent observational evidence of the validity of gen-

eral relativity, from the deflections of radiowaves grazing the Sun¹⁵ and the orbital evolution of binary pulsars¹⁶, suggests that there is no substantial quadrupole moment. Independent evidence for the absence of large asymmetries is provided by the small visible oblateness of the solar surface¹⁷ and by the small rotational splitting of the $l=1$ acoustic modes^{18,19}. This combined evidence suggests that the assumption of a spherical Sun made in the standard model is an adequate approximation in the present context.

The second probe, solar neutrinos, have been detected in ³⁷Cl radiochemical observations¹² and, more recently, in neutrino-electron scattering observations¹³. The fact that detection rates are only about one-third of those predicted by standard solar models has become known as the solar neutrino problem²⁰. Agreement between the two neutrino experiments, which use entirely different techniques, suggests that detection artefacts are not responsible for the problem. Explanations have been attempted both in terms of solar modelling and of neutrino properties. Among the former—essentially attempts to lower the core temperature and hence predicted neutrino production rates—have been higher hydrogen abundance owing to mixing⁹, lower heavy-element abundance²¹ and the possibility of WIMPS increasing the thermal conductivity^{10,11}. The most plausible among the latter is the possible oscillation, resonantly enhanced in matter, between the three species of neutrinos—the Mikheyev-Smirnov-Wolfenstein (MSW) effect²². This would allow electron neutrinos produced in the solar core to be transformed into μ - and/or τ -neutrinos during their passage through the material of the Sun, thus reducing the count rate in the electron-neutrino detectors on Earth.

The third probe is solar seismology. With the benefit of hindsight, oscillations of small portions of the Sun were seen in the early 1960s²³, but probing of the deep interior only became possible when global oscillations were discovered and identified as acoustic eigenfrequencies eleven years ago¹. These modes penetrate deeply into the interior and thereby probe temperature and mean molecular weight of the solar matter, and, more subtly, the rotation, magnetic fields and turbulence of the regions through which they pass. As emphasized in ref. 3, the contribution function is strongly weighted in favour of the surface layers (where the travel time is longest), but the precision of the measurement is so high that core contributions are well over two orders of magnitude greater than the observational errors.

Most models are unable to predict acoustic eigenfrequencies that agree with the data to better than a few microhertz (as compared with experimental uncertainties of substantially less than 1 μ Hz). The discrepancies between model predictions and the observed frequencies of acoustic modes are believed not to be fundamental but to be largely owing to the difficulties of

TABLE 1 Comparison of fine-structure predictions with observation

Reference	Label in Fig. 3	Intercept (μ Hz)	Slope (μ Hz)	Intercept (μ Hz)	Slope (μ Hz)
Data		9.00 (6)	-0.29 (3)	15.6 (1)	-0.46 (6)
5	A	9.38 (1)	-0.296 (4)	16.32 (1)	-0.415 (3)
6	B	9.14 (3)	-0.313 (7)	16.00 (3)	-0.441 (7)
6	C	9.03 (2)	-0.285 (7)	15.80 (1)	-0.410 (7)
7	D	8.77 (3)	-0.295 (7)	—	—
29	E	9.82 (8)	-0.29 (2)	17.1 (1)	-0.40 (2)
11	F	9.36 (8)	-0.426 (2)	15.30 (1)	-0.679 (4)
7	G	6.52 (3)	-0.486 (7)	—	—
7	H	7.88 (3)	-0.387 (7)	—	—
9	I	9.81 (3)	-0.28 (1)	—	—

Straight-line fit of splitting to $(n-21)$ over $n=15-27$. Models A to E are standard models. B and C have different opacities; G and H are models with more or less WIMPS. I is a mixed model. Numbers in parentheses are the 1σ errors in the last significant figure.

modelling the outer layers of the Sun. It is possible to avoid these problems by measuring what is sometimes called the fine structure—the frequency separation δ_{ln} , where l is the degree and n is the order, between nearly coincident modes of l, n and $l+2, n-1$. Modes with low l all have similar behaviour in the outer layers (see, for example, ref. 4), but penetrate more deeply into the core as l decreases. This means that for low- l modes δ_{ln} are nearly independent of the outer layers but are sensitive to the structure of the core. Here we present results for δ_{0n} and δ_{1n} for a range of n .

Until recently, measurements of acoustic (or p-mode) eigenfrequencies and hence of the fine structure have not been of sufficiently high accuracy to provide more than a crude test of theory. Our results enable us to quantify the variation with frequency.

Whole-disk measurements of the solar velocity have been carried out by the Birmingham Solar Seismology group since 1974 using the resonant-scattering technique²⁴. Over the years a considerable amount of solar-velocity data has been accumulated. Here we consider results from measurements made in 1981–1988: combining data from several sites has enabled us to reduce the diurnal sidebands which severely contaminate p-mode fine structure (especially the $l=0, l=2$ difference) in single-site measurements. The data used here are a subset of those used in ref. 25, being two-month stretches of data from one, two or three stations, with a sampling interval of 42 s.

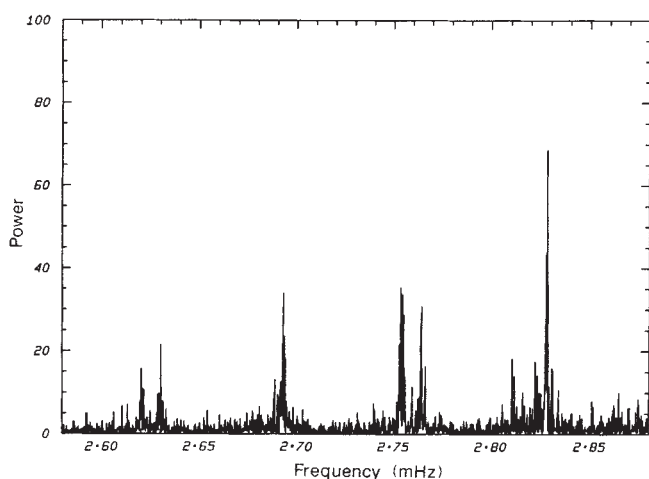
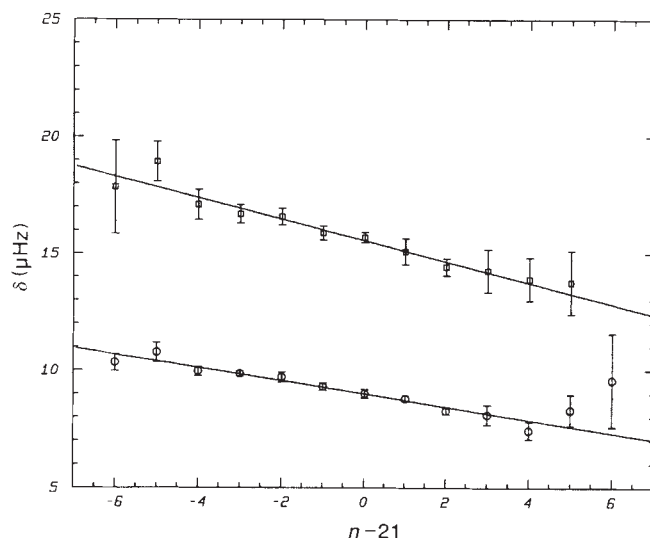


FIG. 1 Section of a typical power spectrum showing fine structure.

FIG. 2 Variation of the fine structure as function of $n-21$; the lower curve is δ_{0n} and the upper curve is δ_{1n} .

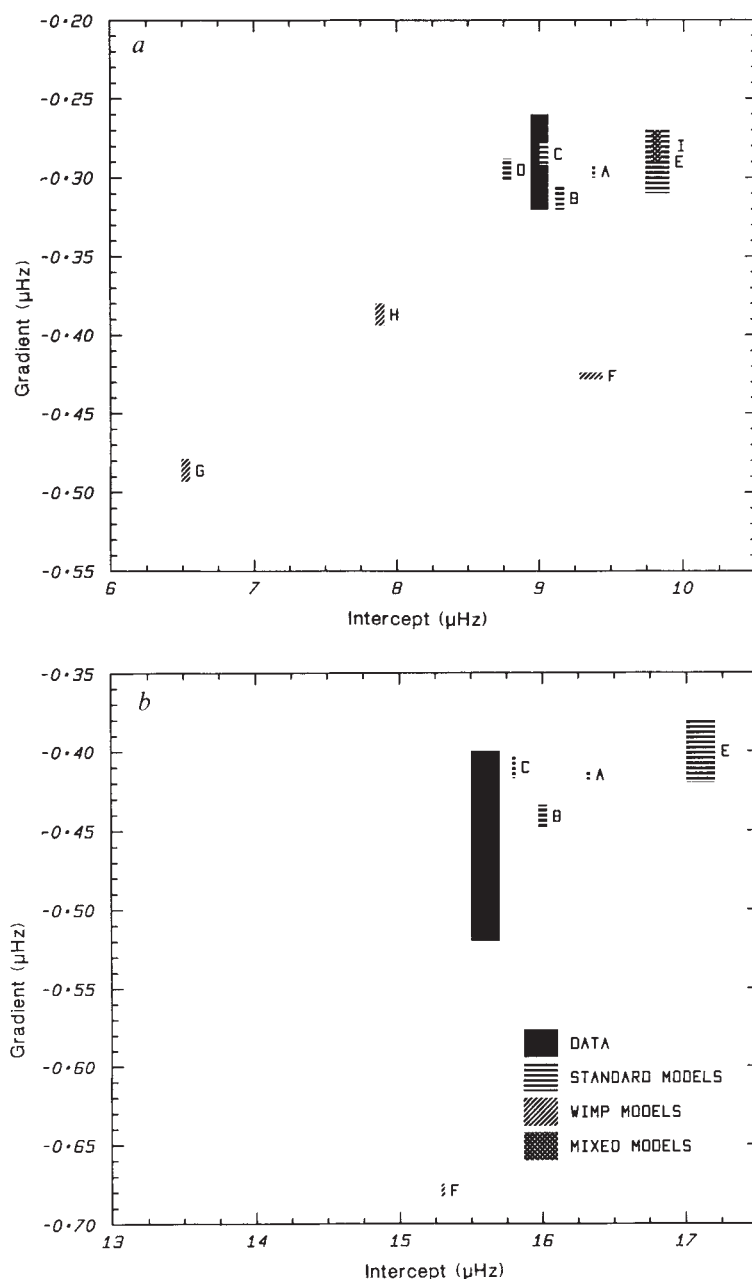


FIG. 3 Comparison of the observations with theoretical predictions. Both are parameterized by an intercept and a gradient of a linear fit. *a* refers to δ_{0n} and *b* refers to δ_{1n} .

A section of a typical power spectrum is shown in Fig. 1. The analysis was the same as that described in ref. 25.

Figure 2 shows the values of the frequency differences δ_{0n} and δ_{1n} as a function of n . The errors shown are the standard deviations of the means of up to eight values: not all modes could be measured in all spectra. In the range covered by the fitted values, these quantities show a significant decreasing trend with increasing n . The data may be fitted by a straight line; no significant improvement in the fits was obtained by using higher-order terms in n .

To test for temporal variation in the fine structure, a straight line was fitted to the values for individual years, in each case giving a gradient and intercept (with the $n-21$ axis) agreeing within errors with those of the mean of all years. Hence, although the individual frequencies vary over the solar cycle²⁵⁻²⁸, we have no evidence of a significant time-dependence of the fine structure.

The gradients and intercepts from the fits are shown in Table 1 and Fig. 3, together with the predictions made by some theoretical models. These models include recent standard models⁵⁻⁷, one mixed model⁹ and WIMP models^{7,29}. The gradients and

intercepts for the models of Christensen-Dalsgaard, Cox *et al.*, Gough and Novotny were calculated from frequencies supplied by them, whereas the others were derived from fine-structure information published in graphical form in the papers referenced. Where no model information is shown for δ_{1n} , no comparable theoretical information is available.

Examination of Table 1 and Fig. 3 shows that, perhaps surprisingly, the agreement between observation and theory is best for recent standard models, the exception being the model of Bahcall and Ulrich²⁹. Four years ago, theorists^{30,31} were proposing that WIMP models gave the best fit to the fine structure. In the intervening years the models have changed (for example, by improvements in the equation of state and, above all, in the numerical precision of the integrations (J. Christensen-Dalsgaard, personal communication)) and so have their predictions in this context. By comparison our data, although of considerably better quality than previous observations³², do lie within the previous error bars.

Although further changes to solar models cannot be ruled out, the success of most current standard models in describing fine structure of the low- l acoustic modes may imply that the

properties of the neutrino are responsible for the solar-neutrino problem. One possible explanation is provided by neutrino oscillations resonantly enhanced in their passage through matter—the MSW effect²². This implies neutrinos of finite mass and thus goes beyond the standard model of the electroweak theory³³. □

Received 6 June; accepted 24 August 1990.

- Claverie, A., Isaak, G. R., McLeod, C. P., van der Raay, H. B. & Roca Cortes, T. *Nature* **282**, 591–594 (1979).
- Deubner, F. L. & Gough, D. O. *A. Rev. Astr. Astrophys.* **22**, 593–619 (1984).
- Bahcall, J. N. *Proc. Int. Conf. on Neutrino Physics and Astrophysics* Vol. 2 (eds Cence, R. J., Ma, E. & Roberts, A.) 253 (University of Hawaii, 1981).
- Ulrich, R. K. & Rhodes, E. J. *Jr Astrophys. J.* **265**, 551–563 (1983).
- Gough, D. O. & Novotny, E. *Sol. Phys.* **128**, 143–160 (1990).
- Christensen-Dalsgaard, J. in *Solar Interior and Atmosphere* (eds Cox, A. N., Livingston, W. C. & Mathews, M.) (University of Arizona Press, in the press).
- Cox, A. N., Guzik, J. A. & Raby, S. *Astrophys. J.* **353**, 698–711 (1990).
- Schatzman, E. & Maeder, A. *Astr. Astrophys.* **96**, 1–16 (1981).
- Lebreton, Y., Berthomieu, G. & Provost, J. *Advances in Helio and Asteroseismology, IAU 123* (eds Christensen-Dalsgaard, J. & Frandsen, S.) 95–98 (Reidel, Dordrecht, 1988).
- Spergel, D. N. & Press, W. H. *Astrophys. J.* **294**, 663–673 (1985).
- Faulkner, J. & Gilliland, R. L. *Astrophys. J.* **299**, 994–1000 (1985).
- Davis, R., Lande, K., Lee, C. K., Cleveland, B. T. & Ullman, J. *Inside the Sun* (eds G. Berthomieu, G. & Cribier, M.) 171–177 (Kluwer, Dordrecht, 1990).
- Hirata, K. S. *et al. Inside the Sun* (eds Berthomieu, G. & Cribier, M.) 179–186 (Kluwer, Dordrecht, 1990).
- Dicke, R. H. & Goldenberg, H. M. *Astrophys. J. Supp.* **27**, 131–182 (1974).
- Robertson, D. S. & Carter, W. E. *Nature* **310**, 572–574 (1984).
- Taylor, J. H. & Weisberg, J. M. *Astrophys. J.* **345**, 434–450 (1989).
- Dicke, R. H., Kuhn, J. R. & Libbrecht, K. G. *Astrophys. J.* **318**, 451–458 (1988).
- Claverie, A., Isaak, G. R., McLeod, C. P., van der Raay, H. B. & Roca Cortes, T. *Nature* **293**, 443–445 (1981).
- Woodard, M. F. *Nature* **309**, 530–532 (1984).
- Bahcall, J. N. *Neutrino Astrophysics* Ch. 1 (Cambridge University Press, 1989).
- Christensen-Dalsgaard, J. *Proc. Symp. Seismology of the Sun and Sun-like Stars* (ed. Rolfe, E. J.) 431–450 (European Space Agency, 1988).
- Bethe, H. A. *Phys. Rev. Lett.* **56**, 1305–1308 (1986).
- Leighton, R. B., Noyes, R. W. & Simon, G. W. *Astrophys. J.* **135**, 474–499 (1962).
- Brookes, J. R., Isaak, G. R. & van der Raay, H. B. *Mon. Not. R. astr. Soc.* **185**, 1–17 (1978).
- Elsworth, Y., Howe, R., Isaak, G. R., McLeod, C. P. & New, R. *Nature* **345**, 322–324 (1990).
- Woodard, M. F. & Noyes, R. W. *Nature* **318**, 449–450 (1985).
- Gelly, B., Fossat, E. & Grec, G. *Astr. Astrophys.* **200**, L29–L31 (1988).
- Palle, P. L., Regulo, C. & Roca Corte, T. *Astr. Astrophys.* **224**, 253–258, (1989).
- Bahcall, J. N. & Ulrich, R. K. *Rev. mod. Phys.* **60**, 297–372 (1988).
- Faulkner, J., Gough, D. O. & Vahia, M. N. *Nature* **321**, 226–229 (1986).
- Däppen, W., Gilliland, R. & Christensen-Dalsgaard, J. *Nature* **321**, 229–231 (1986).
- Jimenez, A. *et al. Advances in Helio and Asteroseismology, IAU 123* (eds Christensen-Dalsgaard, J. & Frandsen, S.) 205–209 (Reidel, Dordrecht, 1988).
- Kuo, T. K. & Pantaleone, J. *Rev. mod. Phys.* **61**, 937–979 (1989).

ACKNOWLEDGEMENTS. We thank all members, past and present, of the Birmingham and Instituto de Astrofísica de Canarias solar oscillations groups for their assistance, and J. Christensen-Dalsgaard, A. N. Cox and D. O. Gough for their comments. This work was funded by SERC and by the CAICYT.

Light-emitting diodes based on conjugated polymers

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CONJUGATED polymers are organic semiconductors, the semiconducting behaviour being associated with the π molecular orbitals delocalized along the polymer chain. Their main advantage over non-polymeric organic semiconductors is the possibility of processing the polymer to form useful and robust structures. The response of the system to electronic excitation is nonlinear—the injection of an electron and a hole on the conjugated chain can lead to a self-localized excited state which can then decay radiatively, suggesting the possibility of using these materials in electroluminescent devices. We demonstrate here that poly(*p*-phenylene vinylene), prepared by way of a solution-processable precursor, can be used as the active element in a large-area light-emitting diode. The

combination of good structural properties of this polymer, its ease of fabrication, and light emission in the green–yellow part of the spectrum with reasonably high efficiency, suggest that the polymer can be used for the development of large-area light-emitting displays.

There has been long-standing interest in the development of solid-state light-emitting devices. Efficient light generation is achieved in inorganic semiconductors with direct band gaps, such as GaAs, but these are not easily or economically used in large-area displays. For this, systems based on polycrystalline ZnS have been developed, although low efficiencies and poor reliability have prevented large-scale production. Because of the high photoluminescence quantum yields common in organic molecular semiconductors, there has long been interest in the possibility of light emission by these organic semiconductors through charge injection under a high applied field (electroluminescence)^{1–7}. Light-emitting devices are fabricated by vacuum sublimation of the organic layers, and although the efficiencies and selection of colour of the emission are very good, there are in general problems associated with the long-term stability of the sublimed organic film against recrystallization and other structural changes.

One way to improve the structural stability of these organic layers is to move from molecular to macromolecular materials, and conjugated polymers are a good choice in that they can, in principle, provide both good charge transport and also high quantum efficiency for the luminescence. Much of the interest in conjugated polymers has been in their properties as conducting materials, usually achieved at high levels of chemical doping⁸, and there has been comparatively little interest in their luminescence. One reason for this is that polyacetylene, the most widely studied of these materials, shows only very weak photoluminescence. But conjugated polymers that have larger semiconductor gaps, and that can be prepared in a sufficiently pure form to control non-radiative decay of excited states at defect sites, can show high quantum yields for photoluminescence. Among these, poly(*p*-phenylene vinylene) or PPV can be conveniently made into high-quality films and shows strong photoluminescence in a band centred near 2.2 eV, just below the threshold for π to π^* interband transitions^{9,10}.

We synthesized PPV (I) using a solution-processable precursor polymer (II), as shown in Fig. 1. This precursor polymer is conveniently prepared from α,α' -dichloro-*p*-xylene (III), through polymerization of the sulphonium salt intermediate (IV)^{11–13}. We carried out the polymerization in a water/methanol mixture in the presence of base and, after termination, dialysed the reaction mixture against distilled water. The solvent was removed and the precursor polymer redissolved in methanol. We find that this is a good solvent for spin-coating thin films of the precursor polymer on suitable substrates. After thermal conversion (typically $\geq 250^\circ\text{C}$, *in vacuo*, for 10 h), the films of PPV (typical thickness 100 nm) are homogeneous, dense and

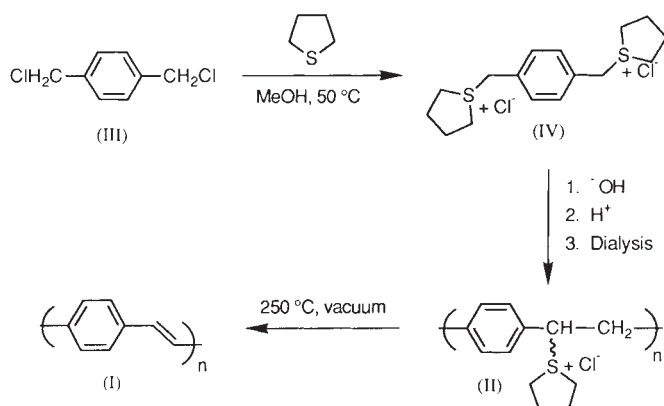


FIG. 1 Synthetic route to PPV.

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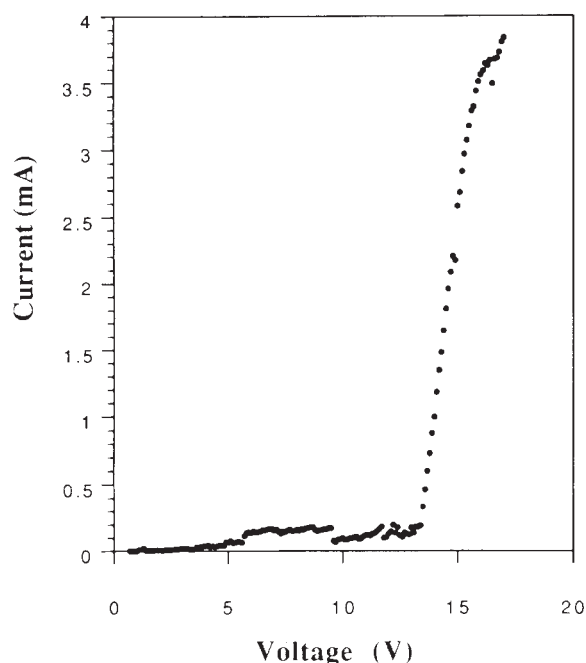


FIG. 2 Current-voltage characteristic for an electroluminescent device having a PPV film 70 nm thick and active area of 2 mm², a bottom contact of indium oxide, and a top contact of aluminium. The forward-bias regime is shown (indium oxide positive with respect to the aluminium electrode).

uniform. Furthermore, they are robust and intractable, stable in air at room temperature, and at temperatures >300 °C in a vacuum¹¹.

Structures for electroluminescence studies were fabricated with the PPV film formed on a bottom electrode deposited on a suitable substrate (such as glass), and with the top electrode formed onto the fully converted PPV film. For the negative, electron-injecting contact we use materials with a low work function, and for the positive, hole-injecting contact, we use materials with a high work function. At least one of these layers must be semi-transparent for light emission normal to the plane of the device, and for this we have used both indium oxide, deposited by ion-beam sputtering¹⁴ and thin aluminium (typically 7–15 nm). We found that aluminium exposed to air to

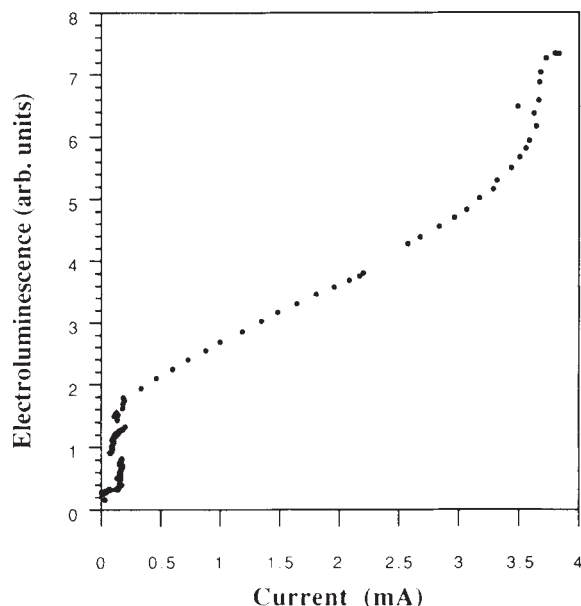


FIG. 3 Integrated light output plotted against current for the electroluminescent device giving the current-voltage characteristic in Fig. 2.

allow formation of a thin oxide coating, gold and indium oxide can all be used as the positive electrode material, and that aluminium, magnesium silver alloy and amorphous silicon hydrogen alloys prepared by radiofrequency sputtering are suitable as the negative electrode materials. The high stability of the PPV film allows easy deposition of the top contact layer, and we were able to form this contact using thermal evaporation for metals and ion-beam sputtering for indium oxide.

Figures 2 and 3 show typical characteristics for devices having indium oxide as the bottom contact and aluminium as the top contact. The threshold for substantial charge injection is just below 14 V, at a field of 2×10^6 V cm⁻¹, and the integrated light output is approximately linear with current. Figure 4 shows the spectrally resolved output for a device at various temperatures. The spectrum is very similar to that measured in photoluminescence, with a peak near 2.2 eV and well resolved phonon structure^{9,10}. These devices therefore emit in the green-yellow part of the spectrum, and can be easily seen under normal laboratory lighting. The quantum efficiency (photons emitted per electron injected) is moderate, but not as high as reported for some of the structures made with molecular materials²⁻⁷. The quantum efficiencies for our PPV devices were up to 0.05%. We found that the failure mode of these devices is usually associated with failure at the polymer/thin metal interface and is probably due to local Joule heating there.

The observation and characterization of electroluminescence in this conjugated polymer is of interest in the study of the fundamental excitations of this class of semiconductor. Here, the concept of self-localized charged or neutral excited states in the nonlinear response of the electronic system has been a useful one. For polymers with the symmetry of PPV, these excitations are polarons, either uncharged (as the polaron exciton) or charged (singly charged as the polaron, and doubly charged as the bipolaron)^{15,16}. We have previously assigned the photoluminescence in this polymer to radiative recombination of the singlet polaron exciton formed by intrachain excitation^{9,10} and, in view of the identical spectral emission here, we assign the electroluminescence to the radiative decay of the same excited state. The electroluminescence is generated by recombination of the electrons and holes injected from opposite sides of the structure, however, and we must consider what the charge carriers are. We have previously noted that bipolarons, the more stable of the charged excitations in photoexcitation and chemical doping studies, are very strongly self-localized, with movement of the associated pair of energy levels deep into the semiconductor gap, to within 1 eV of each other⁹. In contrast, the movement of these levels into the gap for the neutral polaron exciton, which one-electron models predict to be the same as for the bipolaron¹⁵, is measured directly from the photoluminescence

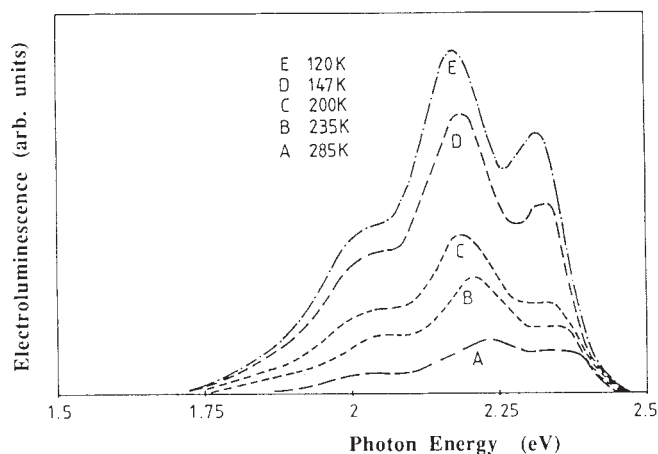


FIG. 4 Spectrally resolved output for an electroluminescent device at various temperatures.

emission to be much smaller, with the levels remaining more than 2.2 eV apart. For electroluminescence then, bipolarons are very unlikely to be the charge carriers responsible for formation of polaron excitons, because their creation requires coalescence of two charge carriers, their mobilities are low and the strong self-localization of the bipolaron evident in the positions of the gap states probably does not leave sufficient energy for radiative decay at the photon energies measured here. Therefore, the charge carriers involved are probably polarons. The evidence that they can combine to form polaron excitons requires that the polaron gap states move no further into the gap than those of the polaron exciton and may account for the failure to observe the optical transitions associated with the polaron.

The photoluminescence quantum yield of PPV has been estimated to be ~8%. It has been shown^{10,17} that the non-radiative processes that limit the efficiency of radiative decay as measured in photoluminescence are due to migration of the excited states to defect sites which act as non-radiative recombination centres, and also, at high intensities, to collisions between pairs of excited states. These are processes that can, in principle, be controlled through design of the polymer, and therefore there are excellent possibilities for the development of this class of materials in a range of electroluminescence applications. □

Received 21 August; accepted 18 September 1990.

1. Vincent, P. S., Barlow, W. A., Hann, R. A. & Roberts, G. G. *Thin Solid Films* **94**, 476–488 (1982).
2. Tang, C. W. & VanSlyke, S. A. *Appl. Phys. Lett.* **51**, 913–915 (1987).
3. Tang, C. W., VanSlyke, S. A. & Chen, C. H. *J. appl. Phys.* **65**, 3610–3616 (1989).
4. Adachi, C., Tokito, S., Tsutsui, T. & Saito, S. *Jap. J. appl. Phys.* **27**, 59–61 (1988).
5. Adachi, C., Tsutsui, T. & Saito, S. *Appl. Phys. Lett.* **55**, 1489–1491 (1989).
6. Adachi, C., Tsutsui, T. & Saito, S. *Appl. Phys. Lett.* **56**, 799–801 (1989).
7. Nohara, M., Hasegawa, M., Hosohawa, C., Tokailin, H. & Kusomoto, T. *Chem. Lett.* 189–190 (1990).
8. Basescu, N. *et al. Nature* **327**, 403–405 (1987).
9. Friend, R. H., Bradley, D. D. C. & Townsend, P. D. *J. Phys.* **D20**, 1367–1384 (1987).
10. Bradley, D. D. C. & Friend, R. H. *J. Phys.: Condensed Matter* **1**, 3671–3678 (1989).
11. Bradley, D. D. C. *J. Phys. D20*, 1389–1410 (1987).
12. Murase, I., Ohnishi, T., Noguchi, T. & Hirooka, M. *Synthetic Metals* **17**, 639–644 (1987).
13. Stenger-Smith, J. D., Lenz, R. W. & Wegner, G. *Polymer* **30**, 1048–1053 (1989).
14. Bellingham, J. R., Phillips, W. A. & Adkins, C. J. *J. Phys.: Condensed Matter* **2**, 6207–6221 (1990).
15. Fesser, K., Bishop, A. R. & Campbell, D. K. *Phys. Rev.* **B27**, 4804–4825 (1983).
16. Brazovskii, S. A. & Kirova, N. N. *JETP Lett.* **33**, 4–8 (1981).
17. Bradley, D. D. C. *et al. Springer Ser. Solid St. Sci.* **76**, 107–112 (1987).

ACKNOWLEDGEMENTS. We thank J. R. Gellingham, C. J. Adkins and W. A. Phillips for their help in preparing the indium oxide films. We thank SERC and Cambridge Research and Innovation Ltd for support.

Tropospheric lifetimes of three compounds for possible replacement of CFC and halons

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CHLORINE and bromine have been implicated in the massive springtime depletion of stratospheric ozone over Antarctica¹. As the source of these halogens is anthropogenic emissions of haloalkanes, the problem has acquired political and economic significance². The chemical industry has been forced to consider urgently possible replacements for conventional halocarbons, and the potential effects on the environment of proposed alternative compounds have been evaluated recently in the Alternative Fluorocarbon Environmental Acceptability Study (AFEAS)³. Three such compounds that are not included in the AFEAS report are CF₂BrH (halon 1201), CF₃CFBrH (halon 2401) and CF₃CF₂CCl₂H (HCFC 225ca). Removal of these compounds from the atmosphere will occur primarily by reaction with the hydroxyl radical, OH (ref. 4). Here we determine, from laboratory studies, the absolute rate of reaction between these three species and OH. Such kinetic data are vital for assessing their viability as replace-

ment compounds. We use these data, along with our measurements of ultraviolet absorption cross-sections, to estimate the tropospheric lifetimes of the halons and HCFC 225ca against removal by OH, and their potential for destroying ozone in the stratosphere. Our approach shows how laboratory measurements can provide a useful first estimate of the environmental acceptability of compounds of this sort.

CF₂BrH and CF₃CFBrH are being considered as substitutes for CF₃Br in fire extinguishers, and CF₃CF₂CCl₂H has useful properties as a solvent. The efficiency with which a unit mass of each halocarbon will destroy stratospheric ozone relative to the most important ozone-depleting molecules CFC-11 (CFCl₃) and CFC-12 (CF₂Cl₂) will depend strongly on the atmospheric lifetime of the compound. The daytime degradation of these hydrogen-containing molecules, RH, will occur primarily by reaction with the OH radical⁴



and it is therefore important to know the rates of the homogeneous gas-phase reactions of OH with RH.

We have determined values of the second-order rate constants, k_1 , for the hydrogen-atom abstraction process (1) and their variation with temperature for the three hydrohalocarbon molecules. Our experimental procedure and data analysis are described fully elsewhere^{5–7}. We used an absolute discharge flow technique with a movable injection point for the reactant to provide time resolution; OH concentrations were measured by resonance fluorescence. The variation of OH concentration with injector position was monitored with a known excess of hydrohalocarbon added. The logarithm of the OH signal was a linear function of calculated contact time, and the gradient was plotted against the concentration of hydrohalocarbon to give a straight line of slope k_1 . Our values for k_1 are given in Table 1 along with the experimental conditions. We used a conventional Arrhenius equation, $\ln k_1 = \ln A - E/RT$, to analyse the temperature-dependence. Thus a plot of $\ln k_1$ as a function of $1/T$ should give a straight line of slope E/R and intercept $\ln A$. Values of the activation energies E in the form E/R and pre-exponential factors A are given in Table 1. Errors on the slope, calculated in a linear least-squares analysis, are the 95% confidence limits. The 95% confidence limits on the intercept were unrealistically large, as is often the case in calculating Arrhenius parameters: we do not quote the errors here. The predictive capacity of the experimental Arrhenius expression is not properly represented by the individual errors on E and A , but rather by the combined expression incorporating both parameters.

We paid particular attention to the possible presence of fast-reacting impurities in the hydrohalocarbon samples, which could seriously affect the measurement of k_1 . All of the

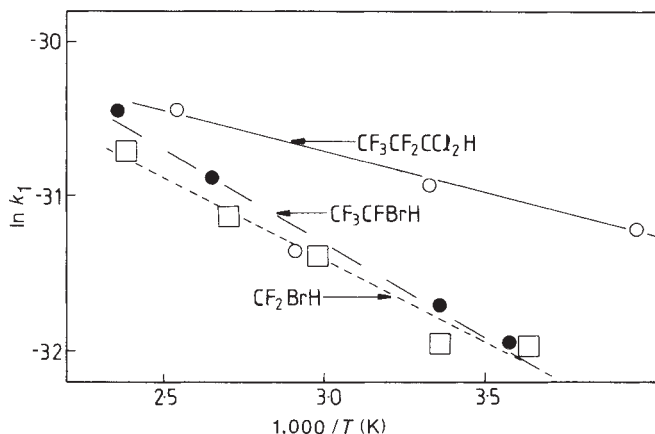


FIG. 1 Arrhenius plots for reactions of RH with OH.

TABLE 1 Kinetic data for reactions with OH

Temperature (K)	Number of runs	Flow-tube pressure (torr)	$10^{-11}[\text{OH}]_0$ (molecule cm^{-3})	$10^{-14}[\text{RH}]$ (molecule cm^{-3})	$10^{14}k_1$ ($\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$)	$10^{13}A$ ($\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$)	E/R (K)
CF_2BrH							
275	6	1.8-1.9	0.7	2.3-10.3	1.3 ± 0.9		
298	11	1.9-2.0	1.7-2.2	3.3-26.0	1.3 ± 0.3		
336	6	1.9	0.7-0.9	3.1-26.8	2.3 ± 0.3	4.4	$1,050 \pm 400$
370	6	1.8-1.9	0.8	2.5-12.8	3.0 ± 0.3		
420	6	1.8-1.9	1.0	2.2-10.9	4.6 ± 0.9		
CF_3CFBrH							
279	7	1.8-1.9	0.8	4.8-24.0	1.3 ± 0.4		
298	12	1.9-4.7	0.9	4.0-59.1	1.7 ± 0.3		
344	6	1.8	0.9	3.2-29.5	2.4 ± 0.5	11.3	$1,250 \pm 350$
377	6	1.8	0.7	1.8-13.6	3.9 ± 0.5		
423	4	1.9	0.6	1.1-5.6	6.1 ± 1.3		
$\text{CF}_3\text{CF}_2\text{CCl}_2\text{H}$							
251	6	1.8-1.9	0.9	2.1-10.0	2.8 ± 1.3		
300	11	1.8-1.9	0.7-0.9	1.8-12.3	3.7 ± 0.8	2.3	550 ± 750
393	7	1.9	0.8-0.9	0.8-4.8	6.1 ± 1.1		

compounds were obtained, with analyses, from ICI Chemicals and Polymers Limited and were used as received. The stated compositions were CF_2BrH 94.23% (major impurities: CF_2ClBr , 3.65%; CF_2HCl , 1.52%; CF_2Cl_2 , 0.19%; $\text{C}_3\text{F}_6\text{H}_2$, 0.05%; $\text{CF}_2\text{HCF}_2\text{Cl}$, 0.4%; C_4F_6 , 50 p.p.m.; C_3F_6 , 10 p.p.m.; $\text{C}_3\text{F}_5\text{H}$, 10 p.p.m.), $\text{CF}_3\text{CFBrH} > 99.5\%$ (major impurities CF_3CFH_2 , CF_2BrH and CF_2ClBr) and $\text{CF}_3\text{CF}_2\text{CCl}_2\text{H} > 99.5\%$ (major impurities HCFC-235 and HCFC-215); the main impurities in the compounds are other saturated halocarbons for which rates of reaction with OH are expected to be similar to those of the compound of interest. The contribution of the saturated species to the rate of loss of OH is thus negligible. Unsaturated impurities can potentially lead to more serious errors, because the addition of OH to an alkene double bond typically occurs at a rate 1,000 times larger than the abstraction of a hydrogen atom from a saturated halogen-containing alkane⁸. The worst case is for CF_2BrH , which has 0.005% of unsaturated impurity. If we assume a rate constant⁸ of $10^{-11} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ for the reaction between a halogenated alkene and OH, then 0.005%

of alkene impurity would contribute 5% to the calculated second-order rate constant. The reactions between OH and the hydrohalocarbon molecules proceed by the abstraction process, reaction (1), and thus their rates are expected to be pressure-independent. We assume, therefore, that the kinetic data gathered in the laboratory at pressures of about 2 torr are also applicable to atmospheric conditions.

If we assume that reaction with OH is the only sink for hydrohalocarbons in the troposphere, then the tropospheric lifetime, τ , is $\{k_1[\text{OH}]\}^{-1}$. Scaling of $\tau_{\text{CH}_3\text{CCl}_3}$ by the ratio of the gas-phase rate constants at 277 K (ref. 9) for the reaction of OH with hydrohalocarbons and CH_3CCl_3 (methyl chloroform, MC)

$$\tau_{\text{RH}} = \tau_{\text{MC}} \times \frac{k_1(\text{MC}, 277 \text{ K})}{k_1(\text{RH}, 277 \text{ K})} \quad (2)$$

as recommended by Prather⁹, circumvents the need for reliable determination of the global mean concentration of OH, a parameter that is poorly established at present. τ_{MC} is better known

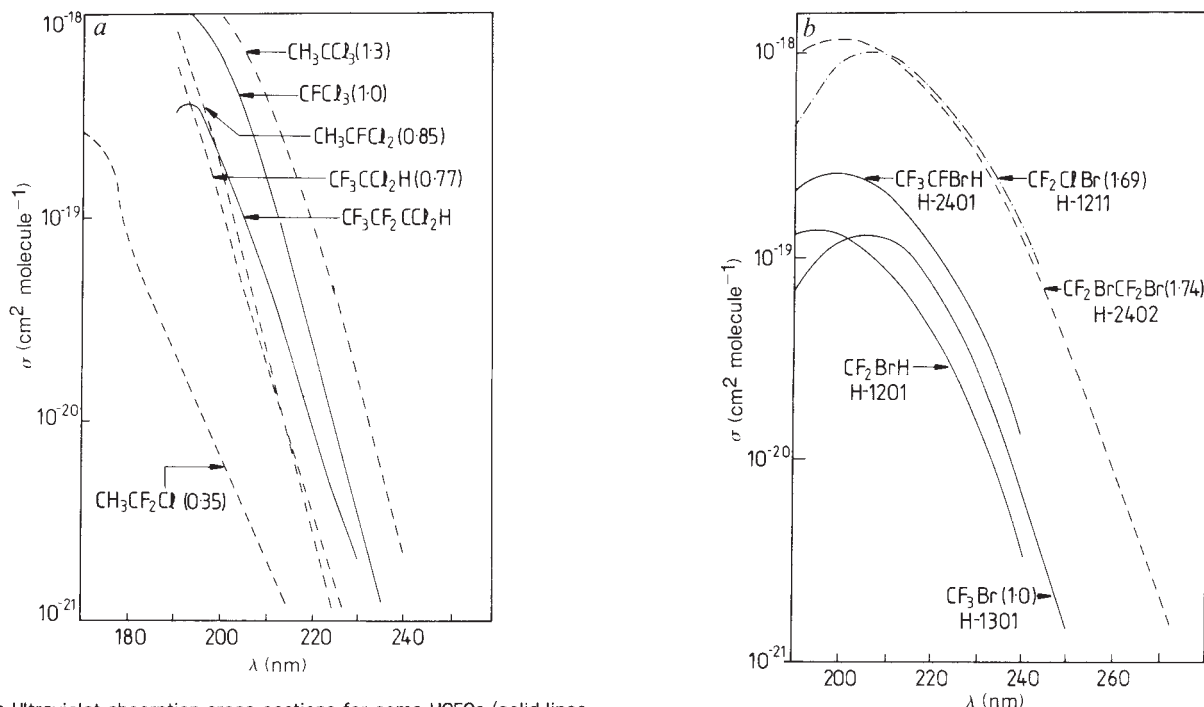


FIG. 2 *a*, Ultraviolet absorption cross-sections for some HCFCs (solid lines, our data; dashed lines, data from refs 15, 16). The numbers in parentheses are the CEFs taken from ref. 17. *b*, Ultraviolet absorption cross-sections for

some brominated halocarbons (solid lines, our data; dashed lines, data from ref. 18). The numbers in parentheses are the BEFs taken from ref. 17.

TABLE 2 Chlorine- and bromine-loading potentials

Compound	$10^{14} k_1$ (277 K) ($\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$)	Lifetime (yr)	CLP or BLP
CF_2BrH	1.0	4.7*	0.07
CF_3CFBrH	1.2	3.9*	0.04
$\text{CF}_3\text{CF}_2\text{CCl}_2\text{H}$	3.2	1.5*	0.01
CFCl_3	—	60†	1.0
CF_3Br	—	72†	1.0
CH_3CCl_3	0.75	6.3†	0.11

* Lifetime calculated from equation (1).

† OSLO two-dimensional model results taken from ref. 14.

than the global concentration of OH because the atmospheric loading of CH_3CCl_3 has been established by field measurements and the only sources are anthropogenic, and documented¹⁰. Again, reaction with OH is assumed to be the only tropospheric sink for CH_3CCl_3 , although Wine and Chameides¹¹ have suggested that hydrolysis of CH_3CCl_3 could contribute as much as 20% to its tropospheric loss. A value of 6.3 yr (ref. 12) for τ_{MC} was used in Prather's work and is the value used here. The estimated uncertainty in τ_{RH} when calculated in this way is approximately $\pm 40\%$.

We use our values of τ_{RH} to determine the atmospheric chlorine-loading potential, CLP, as given by¹³

$$\text{CLP(RH)} = \frac{\tau_{\text{RH}}}{\tau_{\text{CFC-11}}} \times \frac{M_{\text{CFC-11}}}{M_{\text{RH}}} \times \frac{n_{\text{Cl}}}{3} \quad (3)$$

where $M_{\text{CFC-11}}$ and M_{RH} are the molecular weights of CFC-11 and RH, and n_{Cl} is the number of chlorine atoms in the hydrohalocarbon. We use an expression analogous to equation (3) to estimate a bromine-loading potential, BLP, relative to CF_3Br (halon-1301). The second most important factor influencing the potential of a halogenated compound to deplete stratospheric ozone is the dependence on altitude of the release of the active halogen (Cl and Br)¹³, which in turn depends on the action spectrum for photolysis. For example, HCFC-142 has a CLP of 0.159 but an ozone depletion potential, ODP, of 0.05 (Oslo two-dimensional model¹³); the difference arises because HCFC-142 is photolysed in the upper stratosphere rather than lower down where there is a higher concentration of ozone. The ratio ODP/CLP (or ODP/BLP) is termed the chlorine (or bromine) efficiency factor, CEF (or BEF). We have measured the ultraviolet absorption spectra of the three compounds CF_2HBr , CF_3CFBrH and $\text{CF}_3\text{CF}_2\text{CCl}_2\text{H}$ using a commercial spectrophotometer (Perkin-Elmer Lambda 5). The spectra are compared with the spectra of similar substances in Fig. 2a, b. The absorptions of the three compounds are similar to those species for which the CEF or BEF are unity. The ODPs are therefore taken to be equal to the loading potentials for the compounds.

In Table 2, we list estimates of CLPs and BLPs based on our kinetic data. Although the loading potentials are lower for the possible CFC substitutes than for the CFCs themselves by virtue of their more rapid reactions with OH, another piece of information is required to determine the extent to which substitution of hydrohalocarbons for CFCs will lead to a reduction in the transport of chlorinated and brominated species to the stratosphere: to assess fully the implication for pollution of the troposphere and the stratosphere by the substitution of these compounds for CFCs, the fate of their oxidation products must be established. The possible degradation pathways of eight HCFCs and HFCs have recently been reviewed¹⁴. The main products of the oxidation of $\text{CF}_3\text{CF}_2\text{CCl}_2\text{H}$ are likely to be $\text{CF}_3\text{CF}_2\text{CClO}$ and HCl . The degradation of bromine compounds was not considered in the review but products such as CF_2O from CF_2HBr and CF_3CFO from CF_3CFBrH are to be expected. Although the data gathered here are not sufficient for a rigorous

evaluation of the ODPs (which requires detailed modelling), the combination of kinetic and spectroscopic data does provide a reasonable first estimate of the ODPs. □

Received 19 June; accepted 29 August 1990.

- Anderson, J. G. *et al.* *J. geophys. Res.* **34**, 11480–11520 (1989).
- Montreal Protocol on substances that deplete the Ozone Layer, Final Act (United Nations Environment Program, Nairobi, 1987).
- World Meteorological Organisation *Global Ozone Research and Monitoring Project, Rep. No. 20, Scientific Assessment of Stratospheric Ozone 1989 Vol. 2* Appendix: AFEAS Rep. (WMO, Geneva, 1990).
- Derwent, R. G., Volz-Thomas, A. & Prather, M. J. *Global Ozone Research and Monitoring Project, Rep. No. 20, Scientific Assessment of Stratospheric Ozone: 1989 Vol. 2*, Appendix: AFEAS Rep., 123 (WMO, Geneva, 1990).
- Boodaghians, R. B., Canosa-Mas, C. E., Carpenter, P. & Wayne, R. P. *JCS Faraday Trans. 2* **84**, 931–948 (1988).
- Burrows, J. P., Wallington, T. J. & Wayne, R. P. *JCS Faraday Trans. 2* **79**, 111–122 (1983).
- Burrows, J. P., Wallington, T. J. & Wayne, R. P. *JCS Faraday Trans. 2* **80**, 957–971 (1984).
- Atkinson, R. *Chem. Rev.* **86**, 69–201 (1986).
- Prather, M. J. *Global Ozone Research and Monitoring Project, Rep. No. 20, Scientific Assessment of Stratospheric Ozone: 1989 Vol. 2*, Appendix: AFEAS Rep., 147–158 (WMO, Geneva, 1990).
- Midgley, P. M. *Atmos. Envir.* **23**, 2663–2665 (1989).
- Wine, P. H. & Chameides, W. L. *Global Ozone Research and Monitoring Project, Rep. No. 20, Scientific Assessment of Stratospheric Ozone: 1989 Vol. 2*, Appendix: AFEAS Rep., 267–295 (WMO, Geneva, 1990).
- Prinn, R. *et al.* *Science* **238**, 945–950 (1987).
- Fisher, D. A. *et al.* *Global Ozone Research and Monitoring Project, Rep. No. 20, Scientific Assessment of Stratospheric Ozone: 1989 Vol. 2*, Appendix: AFEAS Rep., 299–377 (WMO, Geneva, 1990).
- World Meteorological Organisation *Global Ozone Research and Monitoring Project, Rep. No. 20, Scientific Assessment of Stratospheric Ozone: 1989 Vol. 2*, Appendix: AFEAS Rep., Ch. 6 (WMO, Geneva, 1990).
- Gillotay, D., Simon, P. C. & Brasseur, G. *Planet. Space Sci.* **37**, 105–108 (1989).
- Hubrich, C. & Stuhl, F. *J. Photochem.* **12**, 93–107 (1980).
- World Meteorological Organisation *Global Ozone Research and Monitoring Project, Rep. No. 20, Scientific Assessment of Stratospheric Ozone: 1989 Vol. 1*, 402–410 (WMO, Geneva, 1990).
- Molina, L. T., Molina, M. J. & Rowland, F. S. *J. phys. Chem.* **86**, 2672–2676 (1982).

ACKNOWLEDGEMENTS. We thank the Gassiot Grants Committee of the Meteorological Office (UK) for a studentship for A.C.B., NERC (C.E.C.-M.), the Department of the Environment (A.D.P.), and ICI Chemicals and Polymers Limited for samples of the hydrohalocarbon reactants.

Influence of penetrating solar radiation on the heat budget of the equatorial Pacific Ocean

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SIGNIFICANT interannual variations in global climate, such as the El Niño/Southern Oscillation phenomenon (ENSO), result from interactions between ocean and atmosphere in the tropical Pacific. These interactions are mediated to a large degree by variations in the temperature of the sea surface, particularly in the warm, western portion of the basin^{1–4}. Changes in sea surface temperatures (SSTs) provide precursors for the prediction of climate variability on ENSO timescales. But current ocean-atmosphere models, which estimate SSTs using climatological surface heat fluxes derived from ship observations^{5–7}, consistently overestimate SSTs in the western Pacific by as much as 3 K. Here we use recent satellite observations of ocean transparency, coupled with climatological surface heat fluxes and ocean density profiles, to show that solar radiation in visible frequencies, usually assumed to be absorbed at the sea surface, in fact penetrates to a significant degree to below the upper mixed layer of the ocean which interacts actively with the atmosphere. The net effect is a reduction of the heat input into the upper layer; for a 20-m-thick mixed layer this is equivalent to an annual reduction in temperature of about 5–10 K. Our results provide a natural explanation for the discrepancy between the SSTs predicted by models and those observed.

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The significance of the penetration of solar radiation to the heat budget of the oceanic mixed layer at low latitudes has been suggested theoretically⁸⁻¹⁰ but only recently have the necessary synoptic observations of ocean transparency been available to estimate the magnitude of the associated heat flux and its significance to coupled ocean-atmosphere models of tropical SST. The NIMBUS-7 Coastal Zone Color Scanner (CZCS) made global observations of the colour of the surface ocean¹¹⁻¹³, which is directly related to its transparency to visible solar radiation¹⁴. We used an annual composite of CZCS observations of the equatorial Pacific during 1979 to determine the transparency of the ocean using empirical relationships between satellite-observed radiance from the water and spectral attenuation coefficients with a resolution of 10 nm over the bandwidth 350–700 nm^{11,12,15}. These estimates were determined on a grid bounded by 10° N and 10° S, 120° E and 85° W with a spatial resolution of 5°. Greater temporal resolution was not possible because of insufficient satellite coverage. Solar radiation flux at the sea surface was derived from climatological ship observations on the same grid¹⁶ corrected for albedo and partitioned into the same spectral bands used in the determination of ocean transparency. Net surface heat fluxes, the sum of solar (including contributions from all wavelengths), latent, sensible and radiative terms, were also compiled from the same source. Mixed-layer depths, again with the same spatial resolution, were derived from climatological observations of vertical density distributions¹⁷; the depth of the mixed layer was defined as the depth at which the density had increased by 0.13 kg m⁻³ relative to

the surface value. For each grid point, data were linearly interpolated between standard depths to estimate the mixed-layer depth. We used a density criteria for the mixed-layer depth, rather than the more traditional temperature criteria, because of the large influence of the salinity gradient on the density profile, particularly in the western Pacific^{7,17,18}.

The penetration of surface radiation to the depth of the mixed layer for each wavelength band and at each grid point was calculated from

$$\bar{E}_D(z_m) = \int_{350}^{700} E_D(\lambda, 0^-) \exp(-K(\lambda) z_m) d\lambda$$

where $\bar{E}_D(z_m)$ is the solar irradiance penetrating to the depth of the mixed layer z_m (W m⁻²), $E_D(\lambda, 0^-)$ is the spectral irradiance just below the sea surface (W m⁻² nm⁻¹), $K(\lambda)$ is the spectral attenuation coefficient (m⁻¹), and the integration is carried out over the visible portion of the spectrum. All calculations were carried out at full spatial resolution; for illustrative purposes, data were meridionally averaged.

Mixed layers based on the density difference of 0.13 kg m⁻³ are shallow (<25 m) over much of both western and eastern portions of the equatorial Pacific basin (Fig. 1b). To the east, most of the vertical density variation is due to a shallow thermocline; to the west, a shallow halocline is responsible for the depth of the mixed layer. Deeper mixed layers (>50 m) characterize most of the mid-basin region. This differs from the traditional view of a monotonically increasing mixed-layer depth from the east to the west¹⁹, which is based solely on temperature distributions and does not reflect the strong influence of fresh water on the density structure in the western Pacific. The zonal pattern of solar radiation at the sea surface reflects the convective cloudiness associated with the Walker circulation; the highest radiation fluxes are in the region 150–120° W with lower fluxes to the east and west (Fig. 1a). The ocean transparency is extremely high to the west and is characteristic of pure sea water with little of the biogenic material, phytoplankton pigments and their breakdown products which discolour coastal regions (Fig. 1c). The transparency decreases to the east, where upwelling and a shallow nutricline bring nutrients to the sea surface, stimulating growth and development of higher concentrations of phytoplankton.

These patterns result in a clear zonal variation in the net flux of energy from the base of the surface mixed layer to the layer below (Fig. 2). Penetration rates are high (>25 W m⁻²) in both east and west, and are lower in the mid-Pacific. Errors in the estimate of the net surface heat flux are dominated by systematic errors in the model bulk formulae^{7,20} and are positively correlated with errors in the penetration term because both rely on an estimate of incoming solar radiation. Standard errors in the satellite estimate of the attenuation coefficient are small at these time and space scales (~10%) and contribute relatively little to the error in the magnitude of the penetration term. Given these sources of systematic and random error, we estimate that differences of <15 W m⁻² between the net surface heat fluxes and the flux associated with penetrating solar radiation are not likely to be significant. For most of the basin, the net flux of solar radiation downwards through the base of the mixed layer is equivalent to the climatological net surface heat flux into the ocean.

We conclude that for the equatorial Pacific Ocean, most of the net surface heat flux is lost to the mixed layer as solar radiation penetrates to heat the ocean below the depth of the layer that interacts most strongly with the atmosphere. One of the best defined precursors to six ENSO events has been an increase in SST of ~0.4 K over three months in the western Pacific²¹—the penetrating flux estimated here for the same region, 20 W m⁻², if absorbed in a 20-m layer, would result in a temperature increase of 1.8 K over a comparable time scale.

Current generations of equatorial coupled ocean-atmosphere

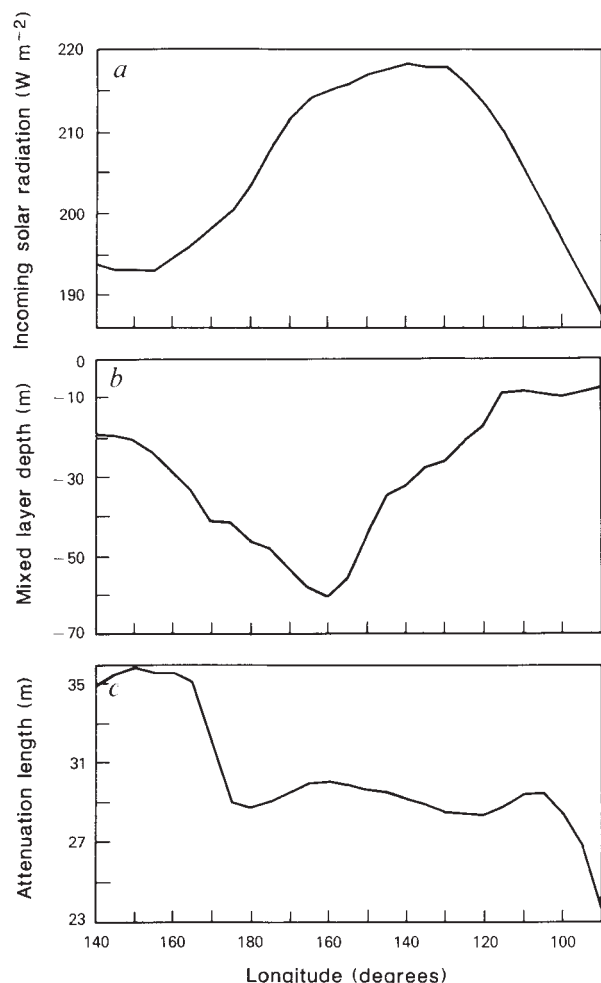


FIG. 1 Zonal patterns over the equatorial Pacific Ocean of *a*, solar radiation, *b*, mixed-layer depth and *c*, satellite-observed attenuation length scales ($1/K$) at 490 nm. Data have been meridionally averaged from 10° N to 10° S and zonally smoothed by a 15° running filter.

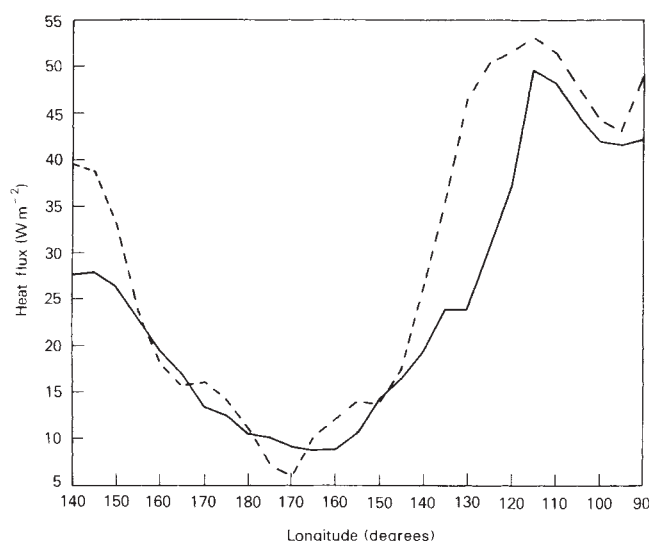


FIG. 2 Zonal patterns in the net flux of radiation from the base of the surface mixed layer (solid) and the net surface heat flux (dotted). Averaging as in Fig. 1.

models produce sea surface temperatures that are several degrees too high when climatological surface heat fluxes, derived from bulk formulae and ship-based meteorological observations, are used, suggesting that the estimates of positive net downward surface heat fluxes contain a systematic error and that the net heat flux into the ocean is close to zero over the western Pacific⁵⁻⁷. Some models of the Pacific warm-water pool²⁰ suggest that high rates of turbulent heat transport in the vertical are required to transport estimated net surface heat fluxes of the order of 20–30 W m⁻² downwards out of the upper mixed layer. Although this turbulent flux may have a role in the region 150° to 115°W (Fig. 2) where there seems to be net heating of the mixed layer, these transports are not likely to be significant in the western Pacific where the shallow salinity 'barrier layer' prevents significant turbulent transport^{7,19,21}. Other models have postulated an error in the formulae for heat flux, and have proposed corrections (increases in cloud attenuation) to force the model to match observed SSTs⁶.

The analysis presented here permits a resolution of this apparent problem. The net radiative transport of heat downward through the base of the mixed layer is approximately equivalent to the estimated climatological net surface heat flux into the ocean over much of the western Pacific. The consequence is that the mixed layer receives effectively a negligible excess of heat over the year as required by the model SST evolution, even given a positive net downward surface heat flux.

One interesting consequence is the sensitivity of SST to variations in the optical properties of the upper ocean in the equatorial Pacific. For example, an increase in phytoplankton concentration in the western Pacific to the level typically observed in the east (0.3 mg chlorophyll m⁻³), results in an increase in the heat trapped in the upper ocean of the order of 10 W m⁻² owing to increased turbidity and absorption of solar radiation. Chlorophyll concentrations of this magnitude were observed in the western Pacific associated with the 1982/1983 ENSO event (Y. Dandonneau, personal communication).

The conclusions drawn here clearly indicate the necessity of considering the heat fluxes associated with penetrating solar irradiance in the development of predictions of tropical Pacific SST variability and its effect on the global climate. Future generations of ocean-observing satellites will be able to make extensive measurements of the surface heat fluxes^{22,23} and the optical transparency²⁴ of the tropical ocean and thus provide an input to future coupled ocean-atmosphere models. □

Received 18 May; accepted 21 August 1990.

- Wyrki, K. *Science* **180**, 66–68 (1973).
- Gill, A. E. *Q. J. R. met. Soc.* **106**, 447–462 (1980).
- Gill, A. E. & Rasmusson, E. *Nature* **306**, 229–234 (1983).
- Cane, M. A. & Zebiak, S. E. *Science* **228**, 1084–1087 (1985).
- Seager, R., Zebiak, S. E. & Cane, M. A. *J. geophys. Res.* **93**, 1265–1280 (1988).
- Blumenthal, M. B. & Cane, M. A. *J. phys. Oceanogr.* **19**, 815–830 (1989).
- Godfrey, J. S. & Lindstrom, E. J. *J. geophys. Res.* **94**, 8007–8017 (1989).
- Lewis, M. R., Cullen, J. C. & Platt, T. *J. geophys. Res.* **88**, 2565–2570 (1983).
- Woods, J. D., Barkmann, W. & Horch, A. *Q. J. R. met. Soc.* **110**, 633–656 (1984).
- Lewis, M. R. *Oceanologica Acta Spec. Iss.*, 91–95 (1987).
- Gordon, H. R., Clark, D. K., Mueller, J. L. & Hovis, W. A. *Science* **210**, 63–66 (1980).
- Brown, O. B. *et al. Science* **229**, 163–167 (1985).
- Feldman, G. C. *et al. Eos* **70**, 634–641 (1989).
- Austin, R. W. & Petzold, T. J. in *Oceanography from Space* (ed. Gower, J. F. R.) 239–256 (Plenum, New York, 1981).
- Smith, R. C. & Baker, K. S. *Limnol. Oceanogr.* **23**, 247–259 (1978).
- Hsuing, J. *J. geophys. Res.* **91**, 10585–10606 (1986).
- Levitus, S. *Climatological Atlas of the World Ocean*, NOAA prof. Pap. 13 (U.S. Government Printing Office, Washington, D.C., 1982).
- Lukas, R. & Lindstrom, E. in *Proc. Aha Huliko'a Hawaiian Winter Workshop on the Dynamics of the Oceanic Surface Mixed Layer* (eds Muller, P. & Henderson, D.) (Hawaii Inst. Geophys., Honolulu, 1987).
- Halpern, D. *Deep Sea Res.* **A27**, 931–940 (1980).
- Nilner, P. & Stevenson, J. *J. mar. Res. (Suppl.)* **40**, 465–480 (1982).
- Rasmusson, E. M. & Carpenter, T. H. *Mon. Wea. Rev.* **110**, 354–384 (1982).
- Liu, W. T. *J. geophys. Res.* **93**, 6749–6760 (1988).
- Liu, W. T. & Gautier, C. *J. geophys. Res.* **95**, 13209–13218 (1990).
- EOSAT/NASA Report of the Joint EOSAT/NASA SeaWiFS Working Group (NASA, Washington, D.C., 1987).

ACKNOWLEDGEMENTS. We thank T. Busalacchi, C. J. R. Garrett, K. Denman, F. Dobson, D. Halpern, D. Hebert, D. Kelly, T. Liu and P. Schopf for comments on an earlier draft, and J. Hsuing for digital heat flux data. We acknowledge support from the Natural Sciences and Engineering Research Council (Canada) and NASA.

Extreme temporal homogeneity of helium isotopes at Piton de la Fournaise, Réunion Island

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OCEAN island basalts (OIBs) have strontium, neodymium and lead isotopic compositions that are different from those of mid-ocean-ridge basalts, (MORBs), reflecting long-term differences in the chemical characteristics of the respective mantle source reservoirs. The high ³He/⁴He ratios at some islands such as Hawaii and Iceland¹⁻⁷ indicate that these basalts come from sources that are less degassed than the source of MORB. Many islands exhibit considerable variability in Sr, Nd and Pb isotopes⁸⁻¹⁰, but detailed studies of temporal variations in helium isotopes have been restricted to Hawaiian volcanoes—at Mauna Loa, for example, significant variations in ³He/⁴He have been found for the past 30,000 years¹¹. Here we report on ³He/⁴He ratios from Piton de la Fournaise volcano on Réunion Island. No variations are found over the long time of 360,000 years, indicating a remarkable uniformity of ³He/⁴He for the (large) mantle source region over this timescale. The He–Sr–Pb systematics at this island may reflect the simultaneous contribution of both recycled materials (perhaps subducted crust) and primitive components to the Réunion source.

Réunion Island (21° 7' S, 55° 32' E) is the present location of the hotspot trace that trends northeast through Mauritius Island, along the Mascarene Plateau toward the Deccan Traps in India (Fig. 1)¹². The total distance of ~5,000 km makes this hotspot chain comparable in length to the Hawaiian Island chain. Such hotspot traces are believed to result from the interaction of a relatively fixed, hot rising plume in the Earth's mantle with the moving lithosphere¹³. Réunion Island is located within the Dupal anomaly, which is a broad, nearly globe-encircling band centred around 30° S^{14,15}. Many islands in this band, including

Réunion¹⁶⁻¹⁹, have characteristically high $^{207}\text{Pb}/^{204}\text{Pb}$, $^{208}\text{Pb}/^{204}\text{Pb}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, reflecting ancient chemical fractionations of U/Pb, Th/U and Rb/Sr. Dupal islands are sometimes thought to contain subducted crust or lithosphere as a mantle component²⁰⁻²³, or to be produced by ancient mantle metasomatism^{23,24}.

Réunion Island has two volcanoes; the extinct volcano Piton des Neiges comprises about two-thirds of the island to the northwest, and the presently active Piton de la Fournaise is located in the southeast. Piton des Neiges forms the basement on which Piton de la Fournaise began to grow sometime before ~350,000 yr ago²⁵. The Piton de la Fournaise eruptions are well documented both historically and chemically and consist of picrites and lavas that are transitional between alkali basalts and tholeiites^{18,19,26,27}. We have measured $^3\text{He}/^4\text{He}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in historic and prehistoric lavas from Piton de la Fournaise. Helium trapped in inclusions was extracted by *in vacuo* crushing of olivine phenocrysts separated by hand-picking under a microscope. Recent lavas studied come from historical eruptions in 1708, 1776 and 1800 AD, and eight others since 1931, including the picritic eruptions of 1931, 1961 and 1977. Prehistoric lavas studied include a basalt dated by ^{14}C on charcoal (455 AD), four basalts dated by U-Th disequilibrium (<7,000, 15,000, 97,000 and 106,000 years old) and two picrites dated by K-Ar (327,000 and 360,000 years old)²⁵.

He isotopes were measured on a mass spectrometer designed and built at the University of California (Santa Barbara) by J. L. The instrument is a 90° curvature, 21-cm radius, statically operated, double-collector mass spectrometer. The sensitivity for He is $>10^{-4}$ A torr⁻¹, and the absolute detection limit is $<10^4$ atoms of ^3He . The inlet system uses a low-temperature (40 K) charcoal trap for separation of He from other rare gases. The precision for He isotopic ratio determinations is very close to the limit based on ion counting statistics for the weak ^3He beam. The total system blank is $\sim 1 \times 10^{-10}$ cm³ STP ^4He . Blanks were always run before samples. A secondary standard of Yellowstone Park gas was always run after samples, with standard size similar to the size of the sample just analysed. This gas has been routinely calibrated against marine air at UCSB and has a $^3\text{He}/^4\text{He}$ ratio of 16.49 ± 0.04 (2σ) R_A (R_A is the atmospheric ratio of 1.39×10^{-6})²⁸. This procedure allows very small samples

to be analysed and a precise check of sample isotopic ratios in the size range of the analysis.

Our He results (Table 1) confirm the earlier observations that Réunion Island is a high $^3\text{He}/^4\text{He}$ hotspot²⁹⁻³³. In addition, the temporal and spatial homogeneity of $^3\text{He}/^4\text{He}$ is remarkable. There has been no discernible change on any timescale between ~1 year and 360,000 years at Piton de la Fournaise (Fig. 2). The He isotope compositions of the picrites are indistinguishable from the basalts. The mean $^3\text{He}/^4\text{He}$ ratio for all samples is $\sim 12.9 \pm 0.4$ (2σ) R_A ; that is, the variation in $^3\text{He}/^4\text{He}$ is only 3% ($n = 20$). This is in marked contrast to the temporal variability in $^3\text{He}/^4\text{He}$ found at Hawaiian volcanoes¹¹.

The constant He isotopic ratios contrast with the petrological and trace-element data, which indicate that the historical picrites are produced by disruption of pre-existing, genetically unrelated olivine-rich cumulates, apparently by pulses of basaltic magmas on a timescale of about 17 years¹⁹. Also, the formation of Dolomieu caldera in 1930, which represented a large change in magma composition¹⁹, was not accompanied by a change in $^3\text{He}/^4\text{He}$. The minor variability in He and Sr isotopic ratios ($^{87}\text{Sr}/^{86}\text{Sr} = 0.70406\text{--}0.70425$; Table 1) indicates that the source region has remained homogeneous during the entire sub-aerial history, and that melting conditions have remained uniform. We can estimate the scale of source homogeneity by assuming that the volumetric production rate of $0.08 \text{ km}^3 \text{ yr}^{-1}$ since 1931²⁶ is grossly representative of the sub-aerial history, and that 7% partial melting of the mantle source is applicable¹⁹. The characteristic length scale for He isotope homogeneity in the mantle source is then $\sim (0.08 \times 360,000/0.07)^{1/3}$, or $>70 \text{ km}$. Constant $^3\text{He}/^4\text{He}$ ratios also suggest short transport times of magma from the source region ($<10^4$ years) and the absence of a long-lived storage reservoir at depth, consistent with inferences based on U-Th disequilibria³⁴. The homogeneity of Sr isotopes is circumstantial evidence that the homogeneity of helium is not due to domination by metasomatic fluids, and also makes it unlikely that external components from the asthenosphere or lithosphere have been added on the way to the surface. Helium is therefore a genuine tracer that is transferred on melting from the source rock to the melt. The $^3\text{He}/^4\text{He}$ record at Piton de la Fournaise is best explained as the result of a well mixed source region, rather than as a mixture of distinct fluid and/or magmatic

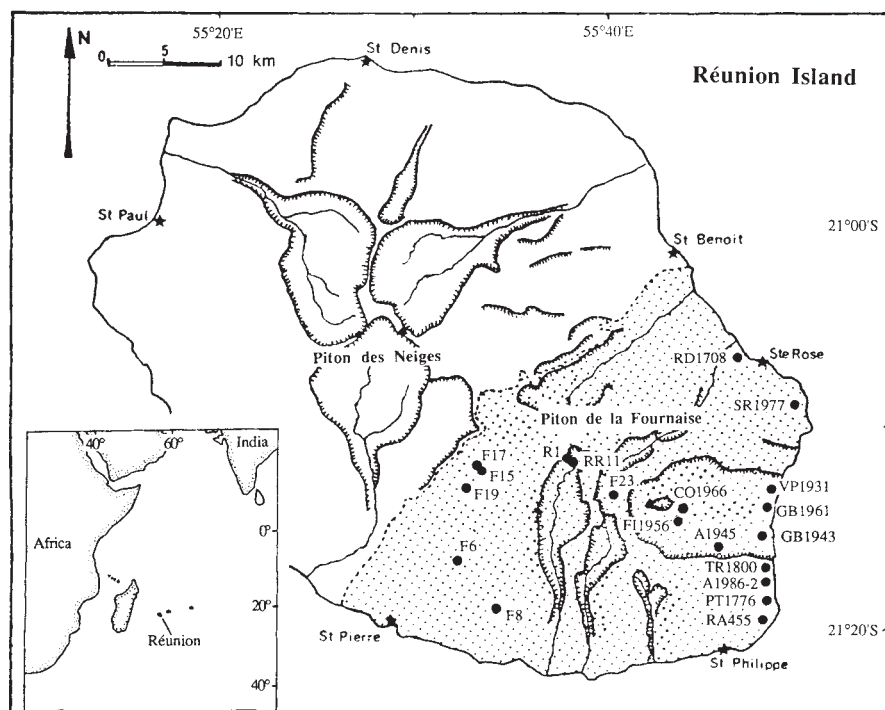
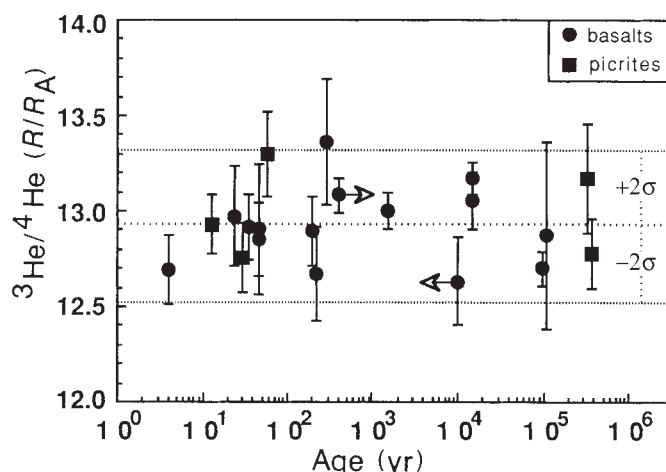


FIG. 1 Sample locations from Piton de la Fournaise, Réunion Island. Inset shows the island location in the Indian Ocean (after ref. 26).

FIG. 2 $^3\text{He}/^4\text{He}$ plotted against age for basalts and picrites from Piton de la Fournaise. The two samples with arrows are plotted at their age limits (a prehistoric flow and a sample which is <7,000 yr old). Where replicate analyses were performed the average result is shown. The dashed lines show the mean value of all the data $\pm 2\sigma$. Error bars for individual samples are $\pm 1\sigma_{\text{mean}}$.



components, which we would expect to show temporal variability.

$^3\text{He}/^4\text{He}$ ratios along mid-ocean ridges are typically between 7–9 R_A . The relatively high $^3\text{He}/^4\text{He}$ ratios at Réunion compared to MORB may indicate some contribution from relatively undegassed mantle, similar to that beneath other hotspots such as Hawaii and Iceland. Réunion is, however, located within the Dupal anomaly. $^3\text{He}/^4\text{He}$ ratios lower than MORB values are found at Dupal islands such as Tristan da Cunha and Gough², consistent with a higher $(\text{U}+\text{Th})/^3\text{He}$ produced by crustal recycling or by mantle enrichment events. In contrast, the

$^3\text{He}/^4\text{He}$ results for Piton de la Fournaise indicate that some Dupal islands can also have 'primitive' He isotope signatures. Castillo³⁵ showed that Dupal anomaly maxima are correlated with low seismic velocities in the lower mantle and with the locations of hotspots. If the relatively high $^3\text{He}/^4\text{He}$ ratios at hotspots such as Hawaii, Iceland and Réunion are evidence for some degree of chemical isolation or layering in the mantle^{36–39}, then the He–Sr–Pb isotope systematics at Réunion also suggest that recycled materials (perhaps slabs) may penetrate into lower-mantle regions, where they may acquire relatively primitive rare-gas signatures but retain much of their enriched Sr and Pb

TABLE 1 He and Sr isotopes in basalts and picrites from Piton de la Fournaise

Sample	Age (years)	Weight (mg)	[He] ($10^{-9} \text{ cm}^3 \text{ STP g}^{-1}$)	$^3\text{He}/^4\text{He}$ (R/R_A)	$2\sigma_{\text{mean}}$	He/Ne He/Ne _{air}	$^{87}\text{Sr}/^{86}\text{Sr}$
Historical samples							
RD1708	282	197.7	4.17	13.36	0.66	18.3	0.70424
PT1776	214	451.3	3.91	12.67	0.48	35.4	0.70419
TR1800	190	210.8	9.37	12.89	0.36	48.0	0.70410
VP1931†	59	363.1	3.28	13.64	0.62	20.1	0.70413
replicate		611.1	6.17	12.96	0.28	65.6	
GB1943	47	135.4	7.06	12.90	0.68	19.2	0.70410
A1945	45	307.6	8.14	12.85	0.38	53.2	0.70413
FI1956	34	174.3	18.1	12.92	0.34	67.6	0.70424
GB1961†	29	496.7	3.78	12.82	0.42	29.8	0.70421
replicate		430.2	8.46	12.69	0.30	74.9	
CO1966	24	179.3	7.96	12.97	0.52	36.5	0.70419
SR1977†	13	464.8	5.41	12.80	0.40	46.8	0.70424
replicate		442.9	15.5	13.06	0.22	120	
A1986-2	4	133.0	13.2	12.69	0.36	51.1	0.70423
Dated samples							
F6*†	360,000 ± 10,000	349.4	3.52	13.17	0.58	27.2	0.70415
F8*†	327,000 ± 20,000	360.0	8.37	12.78	0.36	54.4	0.70422
R1	106,000 ± 16,000	417.0	1.58	12.87	0.99	7.6	0.70406
RR11	97,000 ± 14,000	463.3	32.2	12.70	0.17	243	0.70425
F15	~15,000	419.1	11.2	13.05	0.30	73.1	
F17	15,000 ± 5,000	263.0	46.8	13.17	0.18	236	0.70425
F19	<7,000	178.4	9.08	12.63	0.46	34.4	0.70425
F23	prehistoric	237.0	78.0	13.08	0.17	383	0.70426
RA455	1,535	284.0	30.9	13.00	0.20	173	0.70416

Historical sample identification numbers refer to year (AD) of eruption. Sample localities for historic eruptions are described in ref. 19. F6 and F8 are from near le Tampon, R1 and RR11 from near Nez de Boeuf, F15, F17 and F19 from Plaine des Cafres (Piton Hyacinthe) and F23 from Piton Chisny (Fig. 1). All He results are for olivine mineral separates. Reproducibility of air-standard He sizes is better than 3% (2σ); sample He contents vary much more owing to the variability in abundance of inclusions. All Ne contents were less than twice the blank value and He/Ne ratios should therefore be considered minimum values. Sr isotope results are for whole rocks ($2\sigma < 0.00004$). Sr isotope analyses of historical samples and RA455 were performed at the Centre de Recherches Petrographiques et Geochimiques, some of which were previously reported¹⁹; Sr analyses of dated samples were performed at the Centre de Recherches Volcanologiques. U–Th ages are from Condomines and Bachelery (manuscript in preparation).

* K–Ar ages²⁵.

† Picrite.

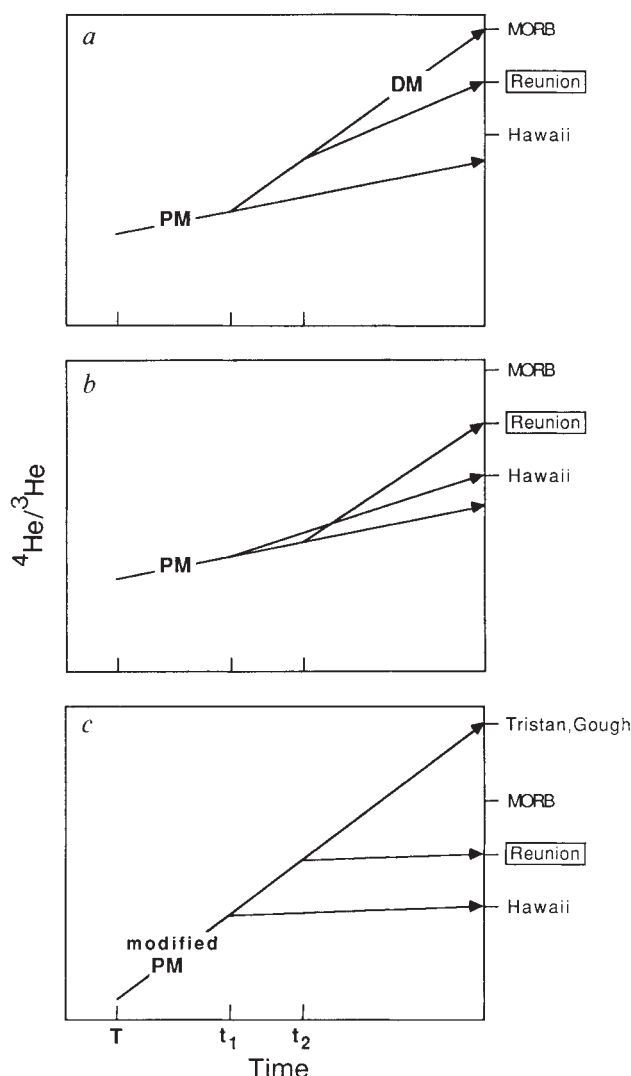


FIG. 3 Simplified evolutionary models for $^4\text{He}/^3\text{He}$ in the Réunion mantle source. Lines shown are schematic because of the nonlinear production rate of ^4He by radioactive decay of ^{238}U , ^{235}U and ^{232}Th . T is the age of the Earth. Relative He isotope compositions for OIBs and MORBs are shown on the right. Melt extraction always leaves a residue with lower Rb/Sr and Th/U. In *a* and *b*, He is assumed to be more incompatible than U and Th during partial melting; in *c* He is less incompatible than U and Th. *a*, Model 1—Réunion source formed at t_2 , by metasomatic enrichment of depleted mantle (DM) with fluids having high Rb/Sr, Th/U and low $(\text{U} + \text{Th})/^3\text{He}$. DM was formed as a residue of melt extraction from primitive mantle (PM) at t_1 . Radiogenic Sr and Pb isotopes at Réunion do not allow the source to be a residue of melt extraction from DM, and the Sr and Nd isotopes in Hawaiian basalts with high $^3\text{He}/^4\text{He}$ are not representative of PM. *b*, Model 2—Réunion source formed as a residue of melt extraction from PM. This decreased Rb/Sr and Th/U, but increased $(\text{U} + \text{Th})/^3\text{He}$ relative to PM. The case shown assumes similar Rb/Sr and Th/U fractionations at t_1 and t_2 ; because the Hawaiian source has lower $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ it would be 'older' than the Réunion source in this case. The case of larger Rb/Sr and Th/U fractionations at t_2 compared to t_1 is also possible, in which case the Réunion source would be formed at t_1 and the Hawaiian source at t_2 . In either case the fractionations in $(\text{U} + \text{Th})/^3\text{He}$ and Rb/Sr are not strictly correlated, because Hawaii has higher $^3\text{He}/^4\text{He}$ and lower $^{87}\text{Sr}/^{86}\text{Sr}$ than Réunion. *c*, Model 3—Réunion source formed as a residue of melt extraction from 'modified PM' at t_2 . Early outgassing produced PM with high $(\text{U} + \text{Th})/^3\text{He}$. Subsequent melt extraction produced a residue with lower Rb/Sr, Th/U and $(\text{U} + \text{Th})/^3\text{He}$. The isotope signature for PM is that of bulk Earth ($^{87}\text{Sr}/^{86}\text{Sr} = 0.7047$ and $^3\text{He}/^4\text{He} = 5 R_A$). As DM also lost U and Th throughout its history whereas PM did not, DM evolved to higher $^3\text{He}/^4\text{He}$ than 'PM', consistent with MORB values of 7–9 R_A . Parent/daughter fractionations are all in the same direction; the magnitudes of the fractionations are also correlated between the Hawaiian and Réunion sources, but not between DM and OIB sources, as DM has the lowest $^{87}\text{Sr}/^{86}\text{Sr}$ but intermediate $^3\text{He}/^4\text{He}$.

isotope signatures. In this case, both primitive and recycled components may contribute simultaneously to the genesis of an individual island such as Réunion.

In addition to the complex mixing described above, other possible explanations for the He–Sr–Pb relations at Réunion include: (1) old enrichment of depleted mantle by fluids having high Rb/Sr, U/Pb, Th/U and low $(\text{U} + \text{Th})/^3\text{He}$; (2) ancient differentiation from primitive mantle by partial melt extraction; (3) ancient differentiation from a remnant of primitive mantle that was outgassed during an early stage of Earth history. These alternatives are qualitatively outlined in Fig. 3. Model 3 leads to the idea that helium may not be as effectively outgassed as is often assumed. In the absence of fluids, such as in the deep mantle, helium is simply partitioned into the melt phase as an incompatible element. Depending on the mineralogical constitution of the source, He might sometimes be more compatible than U or Th because He solubility in solids is not much less than in melts^{40,41}. Under these circumstances partial melting would leave a residue with lower $(\text{U} + \text{Th})/^3\text{He}$, and with time promote increasingly higher $^3\text{He}/^4\text{He}$ ratios in the residual peridotite (harzburgite) compared to more fertile sources. Because melt extraction should also leave a residue with low Rb/Sr and Th/U, the lower $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{208}\text{Pb}/^{204}\text{Pb}$ and higher $^3\text{He}/^4\text{He}$ ratios at islands such as Hawaii and Iceland could suggest that their mantle sources are residues of earlier differentiation of the same reservoir which later produced the Réunion source.

Collectively, the uniform and relatively high $^3\text{He}/^4\text{He}$ ratios coupled with moderately radiogenic Sr and Pb isotopes at Réunion Island can be reconciled with either a simultaneous contribution of recycled and primitive components to the genesis of an individual ocean island volcano, or with metasomatism or ancient differentiation that in some cases produced a decrease in $(\text{U} + \text{Th})/^3\text{He}$ in the mantle. □

Received 24 May; accepted 28 August 1990.

1. Craig, H. & Lupton, J. E. *Earth planet. Sci. Lett.* **31**, 369–385 (1976).
2. Kurz, M. D., Jenkins, W. J. & Hart, S. R. *Nature* **297**, 43–46 (1982).
3. Kurz, M. D., Jenkins, W. J., Hart, S. R. & Clague, D. *Earth planet. Sci. Lett.* **66**, 388–406 (1983).
4. Rison, W. & Craig, H. *Earth planet. Sci. Lett.* **66**, 407–426 (1983).
5. Condomines, M. et al. *Earth planet. Sci. Lett.* **66**, 125–136 (1983).
6. Kurz, M. D., Meyer, P. S. & Sigurdsson, H. *Earth planet. Sci. Lett.* **74**, 291–305 (1985).
7. Poreda, R., Schilling, J.-G. & Craig, H. *Earth planet. Sci. Lett.* **78**, 1–17 (1986).
8. Chen, C.-Y. & Frey, F. A. *J. geophys. Res.* **90**, 8743–8768 (1985).
9. Duncan, R., McCulloch, M. T., Barszczus, H. G. & Nelson, D. R. *Nature* **322**, 534–538 (1986).
10. Gerlach, D. C., Cliff, R. A., Davies, G. R., Norry, M. J. & Hodgson, N. *Geochim. cosmochim. Acta* **52**, 2979–2992 (1988).
11. Kurz, M. D., Garcia, M. O., Frey, F. A. & O'Brien, P. A. *Geochim. cosmochim. Acta* **51**, 2905–2914 (1987).
12. Fisk, M. R. et al. *Geology* **17**, 934–937 (1989).
13. Morgan, W. J. *Geol. Soc. Am. Mem.* **132**, 7–22 (1972).
14. Dupré, B. & Allègre, C. J. *Nature* **303**, 142–146 (1983).
15. Hart, S. R., *Nature* **309**, 753–757 (1984).
16. McDougall, I. & Compston, W. *Nature* **207**, 252–253 (1965).
17. Oversby, V. M. *Geochim. cosmochim. Acta* **36**, 1167–1179 (1972).
18. Fisk, M. R., Upton, B. G. J., Ford, C. E. & White, W. M. *J. geophys. Res.* **93**, 4933–4950 (1988).
19. Albarède, F. & Tamagnan, V. *J. Petrol.* **29**, 997–1030 (1988).
20. McKenzie, D. & O'Nions, R. K. *Nature* **301**, 229–231 (1983).
21. White, W. M. *Geology* **13**, 115–118 (1985).
22. Weaver, B. L., Wood, D. A., Tarney, J. & Joron, J. L. *Geology* **14**, 275–278 (1986).
23. Zindler, A. & Hart, S. R. A. *Rev. Earth planet. Sci.* **14**, 493–571 (1986).
24. Vollmer, R. *Geology* **11**, 452–454 (1983).
25. McDougall, I. *Geochim. cosmochim. Acta* **35**, 261–288 (1971).
26. Bachelery, P. thesis, Univ. Clermont-Ferrand II (1981).
27. Ludden, J. N. *J. Volcan. geotherm. Res.* **4**, 171–198 (1978).
28. Lupton, J. & Graham, D. *Geophys. Res. Lett.* (in the press).
29. Kurz, M. D. thesis, Mass. Inst. Technol./Woods Hole Oceanogr. Inst. (1982).
30. Staudacher, T., Kurz, M. D. & Allègre, C. J. *Chem. Geol.* **56**, 193–205 (1986).
31. Kaneoka, I., Takaoka, N. & Upton, B. J. G. *Chem. Geol.* **59**, 35–42 (1986).
32. Craig, H. & Rison, W. (abstr.) *Eos* **69**, 1144 (1982).
33. Staudacher, T., Sarda, P. & Allègre, C. J. *Chem. Geol.* (in the press).
34. Condomines, M., Hemond, C. & Allègre, C. J. *Earth planet. Sci. Lett.* **90**, 243–262 (1988).
35. Castillo, P. *Nature* **336**, 667–670 (1988).
36. Kaneoka, I. *Nature* **302**, 698–700 (1983).
37. Allègre, C. J., Staudacher, T., Sarda, P. & Kurz, M. *Nature* **303**, 762–766 (1983).
38. O'Nions, R. K. & Oxburgh, E. R. *Nature* **306**, 429–431 (1983).
39. Allègre, C. J. & Turcotte, D. L. *Geophys. Res. Lett.* **12**, 207–210 (1985).
40. Hiyagon, H. & Ozima, M. *Geochim. cosmochim. Acta* **50**, 2045–2057 (1986).
41. Jambon, A., Weber, H. & Braun, O. *Geochim. cosmochim. Acta* **50**, 401–408 (1986).

ACKNOWLEDGEMENTS. We thank P. Bachelery for sampling some of the dated rocks, D. Dautel for performing complementary Sr isotope measurements at CRPG and M. Ort for comments on the manuscript. The He isotope work was supported by the Ocean Sciences Division of the NSF (D.G. and J.L.).

A new biogeographic connection between islands in the Atlantic and Pacific Oceans

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THE affinities between floras of widely separated regions of the world, as well as modes of dispersal of elements in these floras, have long been of interest in evolutionary and phytogeographical studies¹⁻³. While investigating phylogenetic patterns and modes of speciation in the endemic plants of the Juan Fernandez Islands, Chile⁴⁻⁶, we have documented an inter-population separation (disjunction) for the plant genus *Peperomia* (Piperaceae) of more than 5,000 km, one of the longest known in flowering plants. *Peperomia berteroa* ssp. *berteroana* occurs only in the Juan Fernandez Islands in the Pacific Ocean, and *P. berteroa* ssp. *tristanensis* is restricted to the Tristan da Cunha archipelago in the South Atlantic Ocean. Flavonoid and morphological data suggest that plants from Masafuera, the younger of the Juan Fernandez Islands, are ancestral to Tristan da Cunha populations. We propose that long-distance dispersal by birds is the most likely cause of this wide disjunction.

The Juan Fernandez Islands are located 600 km west of continental Chile at about 33° S, 80° W (Fig. 1). The two principal volcanic islands are Masatierra (MT), which is roughly 4 Myr old, and Masafuera (MF), which is 150 km farther west, and 1-2 Myr old⁶. Four distinctive species of *Peperomia* occur in this archipelago: *P. fernandeziana* Miq. (Chilean mainland, MT, MF), *P. margaritifera* Bert. ex Hook. (MT), *P. skottsbergii* C.DC. (MF) and *P. berteroa* Miq. (MT, MF). Comparative morphological studies reveal that the latter species is nearly identical to *P. tristanensis* Christoph., which was treated as a synonym of *P. berteroa* by Christophersen⁷ and Groves⁸ and more recently as two distinct subspecies⁹. *Peperomia berteroa* ssp. *tristanensis* occurs in Inaccessible Island of the Archipelago of Tristan da Cunha, which is in the middle of the South Atlantic Ocean (37° S, 12° W) (Fig. 1). This archipelago is relatively young^{10,11} and consists of three islands: Tristan (0.5 Myr), Inaccessible (2.9 Myr) and Nightingale (18 Myr).

Observations and collections for morphological and chemical studies were made on *Peperomia berteroa* and relatives during three field expeditions to the Juan Fernandez Islands (1980, 1984, 1986). Existing herbarium material was also examined. Twenty-seven morphological characters, including both vegetative and reproductive features, were analysed using numerical taxonomic techniques¹². Leaf-shape, number of principal leaf veins, blade vestiture and spike arrangement showed the most notable discontinuities.

Principal components analysis (Fig. 2) of morphological features reveals *Peperomia berteroa* ssp. *tristanensis* as close to, but clearly distinct from, ssp. *berteroana*. However, of all the populations of ssp. *berteroana* in the Juan Fernandez Islands, some of those from Masafuera are closest morphologically to ssp. *tristanensis* (Fig. 2).

Subspecies were compared for flavonoid compounds sequestered in their leaves. A total of 15 flavones, 5 with sulphate groups, were isolated and identified through high-voltage paper electrophoresis and two-dimensional paper chromatography (Table 1). There is a pattern of island-specific, rather than taxon- or archipelago-specific flavone profiles. The most notable

TABLE 1 Occurrence of flavones in subspecies of *Peperomia berteroa*

Subspecies	Flavones													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<i>berteroana</i> (MT)	+	+	+	+	+	+	+	+	+	+				
<i>berteroana</i> (MF)	+	+	+	+	+	+	+				+	+	+	+
<i>tristanensis</i>	+	+	+	+	+	+	+				+	+	+	+

1, Acacetin; 2, Ac-7-sulphate; 3, Di-7-O-monoglucoside; 4, Di-7-O-rhamnoglucoside; 5, Lu-7-O-monoglucoside; 6, Lu-7-O-diarabinoside; 7, Lu-7-sulphate; 8, Ap-7-glucosidesulphate; 9, Lu-7-O-rhamnoglucoside; 10, Di-7-O-glucoside; 11, Ac-7-glucosidedisulphate; 12, Di-C-arabinoglucoside; 13, Di-7-O-arabinoglucoside; 14, Di-7-sulphate. (Ap, apigenin; Ac, acacetin; Di, diosmetin; Lu, luteolin.)

flavonoid differentiation is seen between plants on Masatierra and those of the Masafuera-Inaccessible island group. Even though the latter two islands belong to two archipelagos separated by more than 5,000 km, plants occurring in them have four compounds in common, none of which is present in the Masatierra populations.

This particular disjunct distribution could be explained by: (1) parallel evolution from a common ancestor in South America; (2) convergent evolution from different ancestors; (3) continental drift; or (4) long-distance dispersal by birds.

The first suggests that the two subspecies would have evolved in parallel from a common ancestral population and would have spread from South America to both archipelagos by means of bird dispersal within the past 3 Myr. This time-frame is suggested by the known ages of the islands where *Peperomia* now grows, and by the slight morphological and chemical differentiation between the Juan Fernandez and Tristan da Cunha populations.

No linking populations, or a common ancestral population with the characteristics shown by the taxa on both islands, have been discovered in South America, despite extensive searches in different herbaria rich in collections of *Peperomia*. Therefore, the ancestor either does not exist in South America, has become extinct (fossils of *Peperomia* are not known from these antecedent localities), or it has not so far been discovered.

The position of the Tristan da Cunha archipelago on the Mid-Atlantic Ridge suggests that there could have been numerous islands that may have served as stepping stones between South America and Africa during the gradual opening of the Atlantic. However, the poverty and general composition of the Tristan flora, as compared with that of South America and Africa, do not support this possibility. Prominent members of the fern floras of both South America and Africa are absent¹³. Migration across archipelagos would be expected to result in a richer flora than the islands have, and one that contains a better

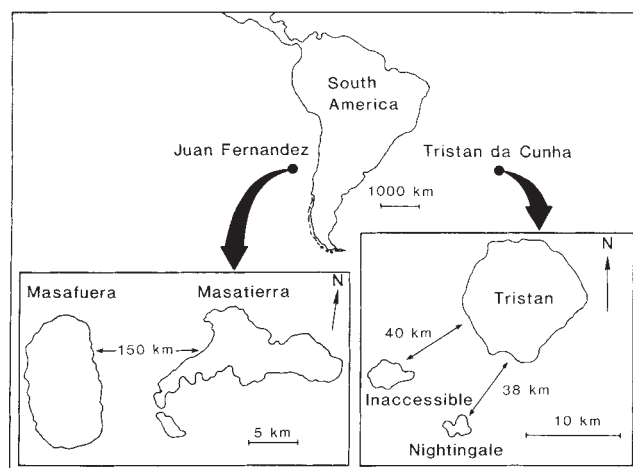


FIG. 1 Location of the Juan Fernandez and Tristan da Cunha archipelagos.

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representation of the prominent elements of the adjacent continents.

That convergent evolution accounts for the disjunct distribution of these two subspecies of *Peperomia* seems unlikely because both taxa are morphologically very similar¹⁴ and sequester a similar array of compounds. This would mean that convergence would have occurred in both morphological and chemical features, which seems improbable.

Continental drift, plate tectonics, and ocean-floor spreading, also seem unlikely to be able to account for the distributional pattern of the subspecies of *Peperomia berteriana*. At the close of the Cretaceous (65 Myr) when the Piperaceae, as well as probably most flowering plant families, were in existence¹⁵, about 800 km probably separated Africa and South America at their closest points, and neither the Juan Fernandez nor Tristan da Cunha Archipelago is older than 18 Myr. Therefore, the Tristan da Cunha Archipelago appeared when the South Atlantic Ocean was already opened to its full extent. Furthermore, the Juan Fernandez Islands have never been connected to continental Chile⁶.

Long distance dispersal of the subspecies of *Peperomia berteriana* seems most concordant with all available information. One characteristic of the genus *Peperomia* that makes it suitable for this mode of dispersal is the production of sticky fruits^{14,16}, which can probably be dispersed among the feathers of migrating birds that visit both archipelagos. Such birds include *Fregetta grallaria* ssp. *melanoleuca* (the Tristan Storm Petrel), which has been recorded as breeding in both archipelagos¹⁷, and *Pterodroma externa* (the Juan Fernandez Petrel), endemic to Masafuera, which probably only touches the Tristan Archipelago accidentally in its circumpolar wanderings. In addition, self-fertilization probably occurs in at least some species of the family Piperaceae^{18,19}, and would be advantageous for island colonization²⁰. Furthermore, there is a marked similarity in the ecology on Tristan da Cunha and the Juan Fernandez archipelago; Masafuera as well as the islands in the Tristan da Cunha group have an alpine and subalpine flora²¹.

The direction of long-distance dispersal seems to have been from Masafuera in the Juan Fernandez Islands to Inaccessible in Tristan da Cunha rather than the reverse. The genus *Peperomia* is represented by four species that are well-distributed on Masatierra and Masafuera; this is not so in Inaccessible where only one rare species exists, known only from the valley leading up from Salt Beach. Phylogenetic relationships based on morphological and flavonoid data²² suggest that the populations on Tristan da Cunha are more derived than those of Juan Fernandez.

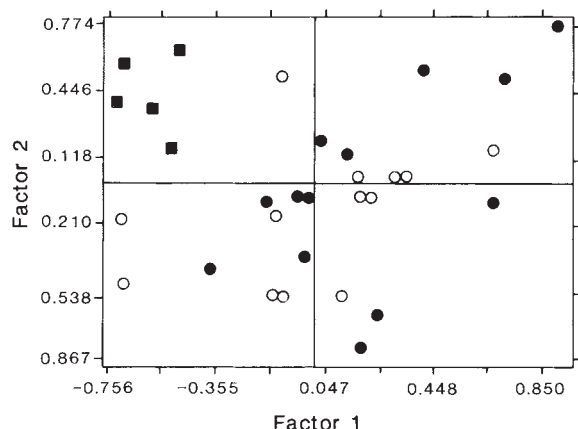


FIG. 2 Principal components analysis of *Peperomia* present in Juan Fernandez and Tristan da Cunha archipelagos. ●, *P. berteriana* ssp. *berteriana* (MT); ○, *P. berteriana* ssp. *berteriana* (MF); ■, *P. berteriana* ssp. *tristanensis*.

Even though several amphitropical Pacific examples in other genera exist (for example, *Rhamphogyne*, Compositae, with one species in the Mascarene Islands and another in New Guinea²³) as well as disjunctions between Hawaii and the central-South Pacific (for example, *Charpentiera*, Amaranthaceae²⁴, and *Tetramolopium*, Compositae^{25,26}), none of these disjunctions is between different oceans. The only other known flowering plant found in both archipelagos (as two different varieties) is *Empetrum rubrum* Vahl. (Empetraceae), which is also found in southern South America²⁷. This example also may be due to long-distance dispersal by birds, but from a continental source²⁷. □

Received 23 April; accepted 1 August 1990.

1. Stevens, P. F. *Notes R. bot. Gdn Edinb.* **30**, 341–359 (1970).
2. Moore, D. M. & Chater, A. O. *Bot. Notiser* **124**, 317–334 (1971).
3. Moore, D. M. in *Taxonomy, Phytogeography and Evolution* (ed. Valentine, D. H.) 115–138 (Academic, London, 1972).
4. Lammers, T. G., Stuessy, T. F. & Silva O., M. *Pl. Syst. Evol.* **152**, 243–266 (1986).
5. Sanders, R. W., Stuessy, T. F., Marticorena, C. & Silva O., M. *Opera Bot.* **92**, 195–215 (1987).
6. Stuessy, T. F., Foland, K. A., Sutter, J. F., Sanders, R. W. & Silva O., M. *Science* **255**, 49–51 (1984).
7. Christophersen, E. in *Results Norw. Scient. Exped. Tristan da Cunha* (ed. Christensen, E.) **55**, 1–29 (Det Norske Videnskaps-Akademi, Oslo, 1968).
8. Groves, E. *Bull. Br. Mus. nat. Hist. (Bot.)* **8**, 333–420 (1981).
9. Valdebenito, H., Stuessy, T. F. & Crawford, D. J. *Brittonia* **42**, 121–124 (1990).
10. Ollier, C. D. *Z. Geomorph.* **28**, 367–382 (1984).
11. Preece, R. C., Bennett, K. D. & Carter, J. R. *J. Biogeogr.* **13**, 1–33 (1986).
12. Rohlf, J., Kishpaugh, J. & Kirk, C. NT-SYS. *Numerical Taxonomy System of Multivariate Statistical Programs* (State University of New York, Stony Brook, 1972).
13. Tryon, A. F. *Br. Fern Gaz.* **9**, 269–276 (1966).
14. Skottsberg, C. *Acta Horti Gotob.* **16**, 251–288 (1946).
15. Raven, P. H. & Axelrod, D. I. *Ann. Mo. bot. Gdn.* **61**, 539–673 (1974).
16. Ridley, H. N. *The Dispersal of Plants Throughout the World* (Reeve, London, 1930).
17. Hagen, Y. in *Results Norw. Scient. Exped. Tristan da Cunha* (ed. Christensen, E.) **20**, 1–237 (Det Norske Videnskaps-Akademi, Oslo, 1952).
18. Martin, F. W. & Gregory, L. E. *Crop. Sci.* **2**, 295–299 (1962).
19. Semple, K. S. *Ann. Mo. bot. Gdn.* **61**, 868–871 (1974).
20. Baker, H. G. *Am. Nat.* **93**, 255–271 (1959).
21. Skottsberg, C. *Nat. Hist. Juan Fernandez Easter Is.* **1**, 193–405 (1956).
22. Valdebenito, H. A. thesis, Ohio State Univ., Columbus (1989).
23. Koster, J. T. *Blumea* **22**, 207–217 (1975).
24. Sohmer, S. H. *Brittonia* **24**, 283–312 (1972).
25. Carlquist, S. *Island Biology* (Columbia, New York, 1974).
26. Lowrey, T. K. *Allertonia* **4**, 203–265 (1986).
27. Moore, D. M., Harborne, J. B. & Williams, C. A. *Bot. J. Linn. Soc.* **63**, 277–293 (1970).

ACKNOWLEDGEMENTS. We thank the many Chilean associates with whom the cooperative studies on the Juan Fernandez flora have been undertaken, especially L. Gaete, A. Landero and E. Ruiz, CONAF of Chile for permission to collect in the islands, especially G. Gonzalez and his associates for aiding our collections; British Museum of Natural History for material (H. N. Hall s.n.) of *Peperomia tristanensis* for flavonoid analysis; and R. Tryon and P. Stevens for critical comments on the manuscript. This work was supported by the NSF and the American Society of Plant Taxonomists.

Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences

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LAKE Victoria, together with its satellite lakes, harbours roughly 200 endemic forms of cichlid fishes that are classified as 'haplochromines'^{1,2} and yet the lake system is less than a million years old. This 'flock' has attracted attention because of the possibility that it evolved within the lake from one ancestral species³ and that biologists are thus presented with a case of explosive evolution. Within the past decade, however, morphology has increasingly emphasized the view that the flock may be polyphyletic^{4,5}. We sequenced up to 803 base pairs of mitochondrial DNA from 14 representative Victorian species and 23 additional African species.

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TABLE 1 Variation in the control region among 32 endemic fishes of the Lake Victoria flock

Species	No. of individuals	Bases at 15 variable positions
1. <i>Astatotilapia piceatus</i>	3	TGGTGGCCATCACCGG
2. <i>Astatotilapia elegans</i>	1	...A...T...T...
3. <i>Astatotilapia nubilis</i>	1	...C...T...T...
4. <i>Ptyochromis sauvagei</i>	3	...C...C...T...
5. <i>Ptyochromis xenognathus*</i>	3	...A...C...T...A
		A A
6. <i>Labrochromis ishmaeli*</i>	3	...T...T...
		C T
7. <i>Platytaeniodus degeni</i>	3	...T...T...
8. <i>Macropodus bicolor</i>	2	...A...C...T...
9. <i>Lipochromis obesus</i>	5	...TG...T...
10. <i>Neochromis nigricans</i>	4	...T...
11. <i>Prognathochromis longirostris</i>	1	...T...
12. <i>Prognathochromis dentex</i>	1	...A...T...
13. <i>Prognathochromis paraguayi</i>	1	...CT...TTA...
14. <i>Harpagochromis guarti</i>	1	C...T...

Variable nucleotide positions are indicated with numbers 1-15 in Fig. 1a; dots indicate identity of a nucleotide with the first species and letters below lines indicate that more than one nucleotide has been observed at this position in the polymorphic species (*). Specimens of species 1, 2 and 4-8 were collected in the Mwanza Gulf, Tanzania (southern Lake Victoria) and the others at Jinja, Uganda (northern Lake Victoria). For species 3, 13 and 14 only positions 84-440 were sequenced. Species 2 and 3 are not strictly endemic to Lake Victoria but also occur in its surrounding lakes, therefore supporting Greenwood's notion of a 'species superclade' of Victoria haplochromines. The species include eaters of algae (7, 10), insects (1, 2, 7), fish larvae (9), fish (11-14) and molluscs (4-6). All endemic haplochromine cichlids of Lake Victoria and the sand-dwelling cichlids of Lake Malawi (group A in Figs 1, 2) were once placed into four monotypic genera plus the genus *Haplochromis*. Greenwood^{19,20} revised the taxonomy of the Lake Victoria haplochromines and assigned ~170 species into 20 endemic genera, reducing the number of species in *Haplochromis* to six. The use of the term 'haplochromines' here is not intended to imply any taxonomic rank assignments to this group of cichlids^{6,14,19,20}.

The flock seems to be monophyletic, and is more akin to that from Lake Malawi than to species from Lake Tanganyika; in addition, it contains less genetic variation than does the human species, and there is virtually no sharing of mitochondrial DNA types among species. These results confirm that the founding event was recent.

Mitochondrial DNA (mtDNA) sequences were obtained from 14 species from 9 endemic genera of the Lake Victoria flock. These included two of the four monotypic genera described by Greenwood⁶ as well as other representatives of the five main ecological and morphological groups, namely eaters of insects, fish, fish larvae, molluscs and algae (Table 1). A 363-base-pair (bp) part of the cytochrome *b* gene and a 440-bp segment of mtDNA that bears part of the threonine transfer RNA gene, all of the proline tRNA gene and the most variable part of the control region were sequenced after enzymatic amplification⁷ (Fig. 1). There is little variation in these mtDNA segments among the 32 specimens examined; only 15 sites out of the 803 surveyed show base-substitutional differences (Table 1). The average number of differences between pairs of the 16 mtDNA types found was less than that occurring within human populations in this part of the control region⁸. A comparable result was evident from electrophoretic comparisons of proteins encoded by nuclear genes^{3,9}. Despite the low genetic variation, there was virtually no sharing of mtDNA types among species (Table 1), a result that is exceedingly unlikely to arise by chance. This finding seems to rule out the possibility that most of the morphological species in Lake Victoria are merely alternative morphs of a few biological species^{10,11}. No length mutations were detected in this survey and no variation was found in either the cytochrome *b* or the tRNA genes. In addition, our findings contrast with studies of other fishes, which generally show more intraspecific variation in conservative regions of mtDNA than is observed in our intergeneric comparisons within Lake Victoria in the control region^{12,13}.

To place these Victorian cichlids in relation to other cichlids, we sequenced both mtDNA segments in 26 additional species (Fig. 1, Table 2). As shown in Table 2, the endemic Victoria

TABLE 2 Base differences in two segments of mtDNA from African cichlid fishes

Species	Geographical origin	No. of base-substitutional differences				
		B	C	D	E	F
A. <i>Hemichromis</i>	W. Africa	55	57	55	55	58
B. <i>Julidochromis</i>	L. Tanganyika	—	40	44	43	41
C. <i>Astatoreochromis</i>	E. Africa	58	—	24	23	32
D. <i>Buccochromis</i>	L. Malawi	63	38	—	7	18
E. <i>Pseudotropheus</i>	L. Malawi	63	38	17	—	18
F. 'Haplochromines'	L. Victoria	63	45	37	36	—

Mean number of differences in a 363-bp segment of the cytochrome *b* gene (from Fig. 1b) appear above the diagonal and in a 440-bp segment bearing tRNA genes and the control region below the diagonal (from Fig. 1a). The cytochrome *b* region was sequenced for a total of 18 specimens, which included 3 from 3 species of the Neotropical genus *Cichlasoma*, 1 *Hemichromis bimaculatus* from West Africa, 1 *Julidochromis regani*, 1 *Astatoreochromis alluaudi* (from Lake Victoria; this species also occurs in rivers of East Africa), 1 *Buccochromis atritaeniatus* (Malawi haplochromine group A), 1 each of 3 species of the *Pseudotropheus tropheops* complex from Lake Malawi (often called 'mbuna', and here called haplochromine group B), and 8 specimens of 7 haplochromine species from Lake Victoria (1, 2 and 4-8 in Table 1). No variation was detected among the specimens from Lake Victoria or among haplochromines of group B from Lake Malawi. The control region was sequenced from 66 specimens, which included 1 *Julidochromis regani* and 1 *Lamprologus birchardi* from Lake Tanganyika (which differ by 53 base substitutions, Fig. 1a), 8 specimens of *Astatoreochromis alluaudi* from Lake Victoria (3 from southern localities in Tanzania and 5 from northern Uganda; no fixed differences were found between northern and southern populations), 9 specimens of 9 species of group A from Lake Malawi (variation not shown), 15 specimens from Malawi of 10 group B species (variation not shown), and the 32 Lake Victoria haplochromines from 14 species whose results appear in Table 1. The Malawi group A species were *Chilotilapia rhoadesi*, *Buccochromis atritaeniatus*, *Nimbochromis polystigma*, *Dimidiochromis compressiceps*, *Maravichromis labiododon*, *Champsocromis spiliorhynchus*, *Protomelas annectens*, 'Haplochromis' *diaboli* and *Sciaenochromis gracilis*¹⁴; the Malawi group B representatives consisted of eight specimens from seven undescribed species of the *Pseudotropheus tropheops* species complex²¹ (P. Reinthal and A. M., unpublished data), 5 of *Pseudotropheus zebra* and 1 each of *Melanochromis auratus* and *Labeotropheus fuelleborni*. Consensus sequences appear in Fig. 1 for *Astatoreochromis* and Malawi groups A and B as well as for the Victoria haplochromines.

haplochromine species, which differ from each other by an average of only 3 substitutions (Table 1), differ from those of Lake Malawi by 54 to 55 substitutions and by at least 77 substitutions from other species. Likewise, the two groups of haplochromines from Lake Malawi, which differ from each other by an average of 24 substitutions, differ by at least 54 substitutions from cichlids elsewhere. Genealogical analyses using *Hemichromis*, from West Africa, as an outgroup confirmed this strong indication of monophyly for the Victoria flock (Fig. 2). Some of the morphologically convergent forms (for example, *Macropodus* from Lake Victoria, and *Chilotilapia* from Lake Malawi) that served before to support the notion of polyphyly⁵ are represented in this study; they confirm our monophyly conclusion. In addition, two species endemic to Lake Tanganyika (*Julidochromis* and *Lamprologus*) are more distantly related to the taxa from Lakes Malawi and Victoria than is *Astatoreochromis*. This nonendemic Victorian haplochromine is the sister group to the endemic cichlid faunas of both Lakes Victoria and Malawi.

The 24 members of the Lake Malawi flock surveyed fall into two monophyletic groups, sand-dwellers and rock-dwellers, here called haplochromine groups A and B, respectively (Fig. 2). As each of these two ecological groups seems to consist of about 200 species¹⁴ and our sampling of the faunal diversity of Lake Malawi may not be adequate, it would be premature to propose the existence of an exact association between phylogenetic position and ecological group for all Malawian species.

T TATAAATCCTCTCTACTGCTTCAAAGAAAGGGATTTTAAACCCCTACCCCTAGCTCCCAAAGCTAAGATCTCTAAGCTAGACTATTTCCTGCCGGGCTCTGCC-TCGATGTAACGTTGTG 120

T LampirologusG.....G.....A.....C.....C.....A.....

E AstatoreochromisGC.....C.....G.....G.....T.....GTC.....T.TT.....CAA.....

M Haplochromine AGC.....C.....G.....A.....T.....GT.....T.TC.....CAA.....

M Haplochromine BGC.....C.....G.....CG.....A.....G.....GT.....T.C.....CAA.....

V HaplochromineC.....C.....G.....A.....G.....T.....GT.....T.....C.....A.....

1

2 3 4

T julidochromis T-CATAAACCATAACTGA-TATAT-CCAACATATATTTAATTAATTAAGCAGATGATTTAAGCCGATACACACCTCTCATACAGTTAAAGATATACCAAG-TACCCACCATCCTATTTCG 360

T lamprologus .A.GTG- .TTT .T.C.A- . . .T.C.CC. .AA. .AG.C. .A. .C-T.T. .T.AA

E astatoreochromis .A.AAC.T.T. .T.A. .- . .G.A. .A. .AG.C. .A. .C-T.A

M haplochromine A .A.CT .T.A. .C- . . .CC. .A. .AG.A. .C. .G. .T.A

M haplochromine B .A.G.TCT .T.A. .CC- . . .AA. .AG.C. .T. .T.T. .A. .

V haplochromine .A.G.CTCT .T.A. .- . .AA. .AG.A. .C-TG.TA

5 6 7 8 9

b
cytochrome *b*

W Hemichromis	CGA	AAA	TCC	CAC	CCC	CTC	CTA	AAA	ATT	GCA	AAC	GAC	GCA	CTA	GTC	GAC	CTC	CCA	ACC	CCC	TCA	AAC	ATC	TCC	ATC	TGA	TGA	AAC	TTT	GGC	TCC	
T Julidochromis	...	...	C...	...	...	...	...	...	...	...	...	...	...	...	T	...	...	...	G...	T	...	...	...	G...	T	...	...	...	...	G...	T	
E Astatoreochromis	...	...	...	...	...	...	...	...	...	...	...	...	...	...	T	T	...	...	G...	T	...	T	...	T	G...	T	...	...	...	...	A...	T
M Haplochromine A	...	...	A...	...	...	...	...	...	...	...	...	...	...	...	T	T	...	...	G...	T	...	...	T	T	G...	T	...	...	...	...	T	T
M Haplochromine B	...	...	A...	...	...	...	...	...	...	...	...	...	...	...	T	T	...	...	G...	T	...	...	T	T	G...	T	...	...	...	...	A...	T
V Haplochromine	...	...	G...	...	...	...	...	...	...	...	A...	...	...	...	T	T	...	...	C...	G...	T	...	...	T	G...	T	...	...	...	...	T	T

W Hemichromis	CTC	CTC	GGA	CTT	TGC	CTT	ATT	GCT	CAA	ATC	CTG	ACA	GGC	CTC	TTC	CTA	GCA	ATA	CAC	TAC	ACC	TCT	GAT	ATC	ACC	ACA	GCA	TTC	TCA	TCC	GTT
T Julidochromis	..A	..A	...	..C	..T	..C	...	..A	...	..G	GAT	...	..T	...	...	..C	...	...	..T	...	..C	..C	...	G...	..C	..C	..T	..C	...	..C	
E Astatoreochromis	..G	..A	...	..C	..T	..A	G..C	..C	...	...	GAT	...	..T	...	...	..C	...	...	..A	...	..C	...	...	G...	..C	..C	..T	..C	...	A..C	
M Haplochromine A	..A	..A	...	..G	..C	..T	..A	GC	..C	...	...	...	..T	...	...	..C	...	...	...	...	..C	...	...	G...	..C	..C	..T	..C	...	A..A	
M Haplochromine B	T..A	..A	...	..G	...	..T	..A	GC	..C	...	...	...	..T	...	...	..C	...	...	...	...	..C	...	...	G...	..C	..C	..T	..C	...	A..A	
V Haplochromine	..A	T..A	...	..T	..C	..T	..A	GC	A..C	...	...	...	..T	...	...	..C	...	...	...	...	..C	...	...	G...	..C	..C	..T	..C	...	..C	

W Hemichromis	GCT	CAC	ATT	TGT	CGA	GAC	GTA	AAT	TAC	GGC	TGA	CTC	ATC	CGA	AAC	ATA	CAT	GCC	AAC	GGC	GCA	TCC	TTC	TTC	TTT	ATC	TGT	ATT	TAC	CTT	CAC
T Julidochromis	..C	...	..C	...	..T	...	..C	..C	...	..T	...	...	...	...	..G	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E Astareochromis	..C	...	...	...	..T	...	..T	..C	...	...	...	...	...	..T	...	..G	..C	...	...	...	...	..T	...	...	...	...	..C	..T	...	...	...
M Haplochromine A	..C	...	...	..C	..T	..T	..C	...	...	...	...	..T	...	...	..G	..C	...	...	...	...	..T	...	...	...	...	...	..C	..T	...	...	...
M Haplochromine B	..C	...	..C	..C	..T	..T	..C	...	...	...	...	..T	...	...	..G	..C	..T	...	...	...	...	..T	...	...	...	...	..C	..T	...	...	...
V Haplochromine	..C	...	..C	..C	..T	..T	..C	...	...	...	...	..T	...	...	..G	..C	..T	...	...	...	...	..T	...	...	...	...	..C	...	...	...	..C

W Hemichromis	ATT GGC CGA GGA CTA TAC TAC GGT TCA TAC CTC TAT AAA GAA ACA TGA AAC ATC GGA GTC ATC CTC CTT CTT CTA ACA ATA ATA
T Julidochromis	G T... ..T ..C ..C ..T T A... ..AAA GAA ..CT ..TTCTT
E Astatoeochromis	..CCTCCCCCTATCTTG ..AT
M Haplochromine A	..CCGC ..CCCCTATACTTG ...
M Haplochromine B	..CCGC ..CCCCTATACTT
V Haplochromine	..CT ..G... ..C ..CCT ..TATACTT

Cichlasoma congeners or with *Hemichromis*, from West Africa, for the African taxa. Blanks, missing sequence; N, Neotropics; T, Lake Tanganyika; E, East Africa; M, Lake Malawi; V, Lake Victoria.

METHODS. DNA was extracted from tissues of frozen or ethanol-preserved specimens^{7,8}. Amplifications were performed in 25- μ l volumes of Tris buffer (67 mM, pH 8.8) containing 2 mM MgCl₂, 1 mM of each dNTP, 1 μ M of each primer, 10–1,000 ng template DNA and *Taq* polymerase (1 unit; Cetus). The primers used for the cytochrome *b* gene were L14724 (5'-CGAAGCTTGATAT-GAAAAACCATCGTTG-3', designed by S. Pääbo) and H15149 (ref. 7); for the control region they were L15926 (ref. 7) and H16498 (5'-CCTGAAGTAGGAAC-CAGATG-3'). L and H refer to the light and heavy strands, respectively, and the numbers refer to the position of the 3' base of the primers in human mtDNA²². The temperature profile for 30 cycles of double-stranded and 35 cycles of single-stranded amplification was 45 s at 94 °C (denaturation), 1 min at 55 °C (annealing of primers), 2 min at 72 °C (polymerization). The rest of the protocol is reported in detail in ref. 7.

The patterns of base changes in the two regions sequenced behave as expected from surveys of other groups of vertebrates. Transitions outnumber transversions in both segments, and silent changes outnumber replacement changes in the cytochrome *b* gene. Moreover, the rate of change in the control region exceeds that in the cytochrome *b* gene. The results give no reason to suppose that the rates of mtDNA evolution are uneven or accelerated among East African cichlid fishes.

The likelihood of extreme recency for the Victorian flock is underscored by the finding that species within the Neotropical genus *Cichlasoma* differ by as much as 11% in their cytochrome *b* sequences⁷ (Fig. 1b), whereas the Victoria flock differs by only 5% from the Malawi flock. Indeed, no diversity in the cytochrome *b* segment (legend to Table 2) was evident within the Victoria flock. An estimate of the divergence times among the flocks is based on the assumption that the mean rate of divergence in the cytochrome *b* gene is 2.5% per million years. Our molecular results date the origin of the extant Victorian flock at less than 200,000 years ago (Fig. 2). Lake Victoria's age has been estimated to be between 250,000 and 750,000 years¹⁵, but younger ages are considered likely for the present-day fauna³. This date and the other estimated divergence times (Fig. 2) fit with the estimated geological ages of the three lakes (1–2 million years (Myr) for Lake Malawi and 2–4 Myr for Lake Tanganyika¹⁶). So it is likely that the Victoria flock arose within the lake.

Furthermore, this result weakens the hypothesis that similarly specialized species from different lakes are more closely related

to each other than to morphological generalists from the same lake. These specializations probably evolved repeatedly and independently. The establishment of the monophyly of this species flock draws attention to the remarkable speed of the morphological diversification in Lake Victoria without an acceleration of molecular evolution.

Unfortunately, we are losing the opportunity to study the cichlid fauna of Lake Victoria, because much of it is going or has gone extinct as a result of the introduction of a non-endemic predatory fish.^{17,18} □

Received 30 July; accepted 20 August 1990.

1. Fryer, G. & Iles, T. D. *The Cichlid Fishes of the Great Lakes of Africa. Their Biology and Evolution* (Oliver & Boyd, Edinburgh, 1972).
2. Regan, C. T. *Proc. zool. Soc. Lond.* **1922**, 157–191 (1922).
3. Sage, R. D., Loiselle, P. V., Basasibwaki, P. & Wilson, A. C. in *Evolution of Fish Species Flocks* (eds Echelle, A. A. & Kornfield, I.) 185–201 (University of Maine Press, Orono, 1984).
4. Greenwood, P. H. *Bull. Br. Mus. nat. Hist. (Zool.)* **44**, 249–290 (1983).
5. Greenwood, P. H. *Bull. Br. Mus. nat. Hist. (Zool.)* **45**, 209–231 (1983).
6. Greenwood, P. H. *Bull. Br. Mus. nat. Hist. (Zool.)* **3**, 295–333 (1956).
7. Kocher, T. D. *et al. Proc. natn. Acad. Sci. U.S.A.* **86**, 6196–6200 (1989).
8. Vigilant, L., Pennington, R., Harpending, H., Kocher, T. D. & Wilson, A. C. *Proc. natn. Acad. Sci. U.S.A.* **86**, 9350–9354 (1989).
9. Nei, M. & Roychoudhury, A. K. *Evol. Biol.* **14**, 1–59 (1982).
10. Sage, R. D. & Selander, R. K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4669–4673 (1975).
11. Meyer, A. *Evolution* **41**, 1357–1369 (1987).
12. Avise, J. C., Bermingham, E., Kessler, L. G. & Saunders, N. C. *Evolution* **38**, 931–941 (1984).
13. Thomas, W. K. & Beckenbach, A. T. *J. molec. Evol.* **29**, 233–245 (1989).
14. Eccles, D. H. & Trewavas, E. *Malawian Cichlid Fishes. The Classification of Some Haplochromine Genera* (A. W. Diekhoff Lake Fish Movies, Herten, 1989).
15. Temple, P. H. *Biol. J. Linn. Soc.* **1**, 363–371 (1969).
16. Banister, K. E. & Clarke, M. A. *J. nat. Hist.* **14**, 483–542 (1980).
17. Barel, C. D. N. *et al. Nature* **315**, 19–20 (1985).
18. Miller, D. J. *Trends Ecol. Evol.* **2**, 56–59 (1989).
19. Greenwood, P. H. *Bull. Br. Mus. nat. Hist. (Zool.)* **35**, 265–322 (1979).
20. Greenwood, P. H. *Bull. Br. Mus. nat. Hist. (Zool.)* **39**, 1–101 (1980).
21. Ribbink, A. J., Marsh, B. A., Marsh, A. C., Ribbink, A. C. & Sharp, B. J. S. *Afr. J. Zool.* **18**, 149–310 (1983).
22. Anderson, S. *et al. Nature* **290**, 457–465 (1981).
23. Gilbert, T. L. *et al. Nucleic Acids Res.* **16**, 11825 (1988).
24. Johansen, S., Guddal, P. H. & Johansen, T. *Nucleic Acids Res.* **18**, 411–419 (1990).
25. Swofford, D. L. *PAUP: Phylogenetic Analysis Using Parsimony, Version 3* (Illinois Natural History Survey, Champaign, Illinois, 1989).
26. Felsenstein, J. *Evolution* **39**, 783–791 (1985).
27. Kornfield, I. L. *Experientia* **34**, 335–336 (1978).
28. Kornfield, I. L., McKaye, K. & Kocher, T. *Iszyme Bull.* **18**, 76 (1985).
29. Irwin, D. M., Kocher, T. D. & Wilson, A. C. *J. molec. Evol.* (in the press).

ACKNOWLEDGEMENTS. We thank K. Barel, R. Hoogendoorn, I. Kornfield, P. Reinthal, M. Stiassny and F. Witte for the collection and identification of specimens, I. Kornfield and P. Moran for mtDNAs of six Malawian cichlids and D. Irwin, I. Kornfield, M. Nishida, P. Reinthal and M. Stiassny, and especially E. Prager, for discussion. This work was supported by the NSF and NIH (A.C.W.), a Sloan fellowship to A.M., and an NIH fellowship to T.D.K.

Transparency and the uniqueness constraint in human and computer stereo vision

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THE sensation of depth that is obtained with human binocular vision results from the differences in the projection of the world onto the two retinæ. The process entails solving the problem of stereo correspondence, which involves choosing the correct matches between left and right image features. Many computational models of stereo vision assume a uniqueness constraint on stereo matching—that is, each feature identified in one image should eventually be matched with only one feature in the other image^{1,2}. This constraint would seem to be justified, as allowing non-unique matches would be tantamount to supposing that the scene entities to which matches relate are in two places at once¹. The value of the uniqueness constraint for eliminating false matches has been demonstrated in a variety of stereo algorithms. Yet on the basis of psychophysical results Weinshall³ concluded that it was not used by humans in dealing with certain types of ambiguous random-dot stereograms. We have now tested how Weinshall's stereograms are dealt with by PMF⁴, a stereo algorithm which uses a

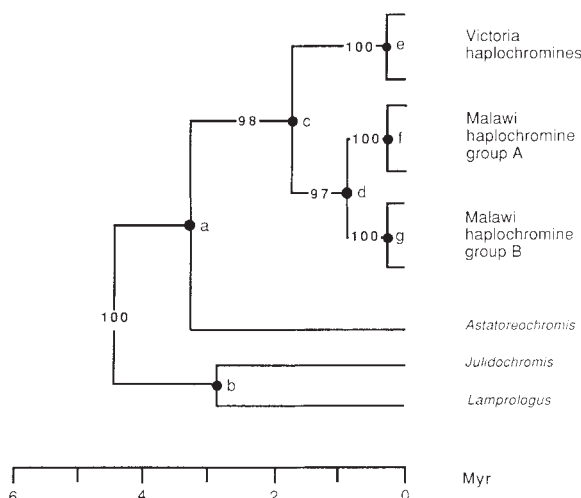


FIG. 2 Evolutionary tree for 36 African cichlid species based on a parsimony analysis²⁵ of mtDNA sequences. The tree is a composite based on two approaches. Only amino-acid replacement changes in cytochrome *b* were used to confirm the two deepest nodes, a and b, as well as to place the root on the ab branch, using *Hemichromis* as an outgroup. The four equally short trees obtained (each with 14 steps, consistency index CI=0.75) did not sort out the branching order inside the group stemming from node a. A second approach used control region data with two cichlids of Lake Tanganyika (*Julidochromis* and *Lamprologus*) as the outgroup and established the branching pattern shown, with high boot-strap values (97–100)²⁶ confirming each of the internal branches (ab, ac, cd, ce, df and dg). The data did not allow the resolution of the relationships within any of the three assemblages stemming from nodes e, f and g (accordingly, parsimony analysis yielded nine equivalent solutions, each requiring 178 mutations; CI=0.826). Other studies suggest that the cytochrome *b* gene diverges at a rate of at least 2.5% per million years in mammals²⁹. On the basis of this rate and the per cent differences obtainable from Table 2, *Astatoreochromis* shared a common ancestor with the endemic Lake Victoria cichlids ~3.5 Myr ago, in exact agreement with the date inferred from studies of proteins encoded by nuclear genes³. The two main groups of cichlid fishes from Lake Malawi seem from this approach to have had a common ancestor ~700,000 years ago, the geological age of this lake being 1–2 Myr¹⁶. The low genetic distances observed among the proteins of the Malawi haplochromines B are consistent with this inference^{27,28}.

unique-matches selection procedure in conjunction with a purely local similar-disparity support scheme. We found that PMF produces results that are closely analogous to the psychophysical results. This suggests that Weishall's experiments should not be interpreted as evidence that the human stereo mechanism establishes non-unique matches.

With the PMF algorithm, support for each potential match is sought within a local neighbourhood from other potential matches that share similar disparities. Similarity is defined in terms of the disparity between potential matches not exceeding

a moderate disparity gradient limit⁵. (Psychophysical measurements suggest that the human visual system is limited by a disparity gradient of about unity for allowable binocular fusion⁶. We exploited the disparity gradient concept to establish stereo correspondences. We view⁵ the magnitude of the disparity gradient limit in PMF's local support scheme in terms of a trade-off between disambiguation power and the ability to deal with as wide a class of scene structures as possible. Gradient limits less than unity provide greater disambiguating power, and are often therefore preferable for finding matches, but at the

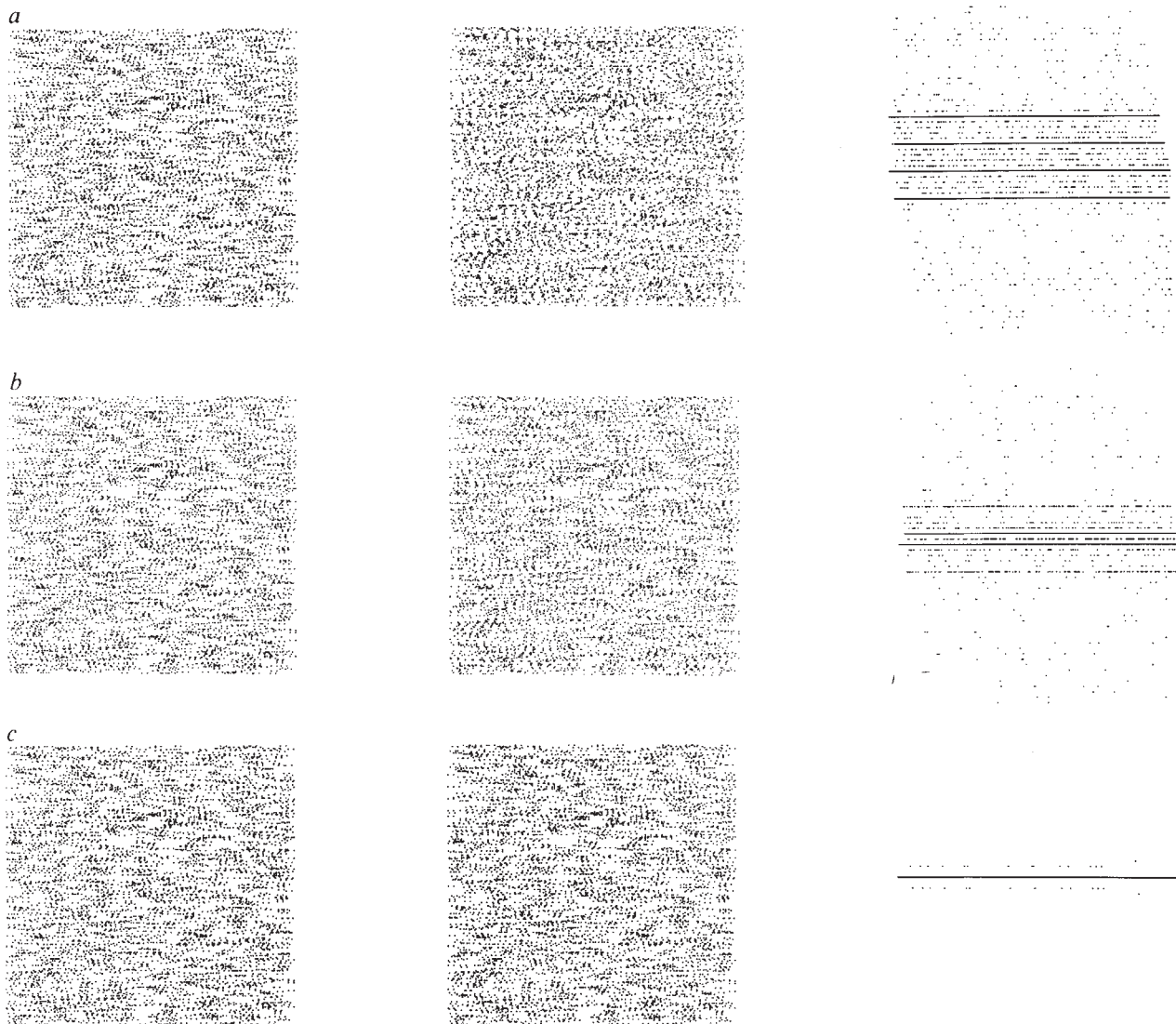
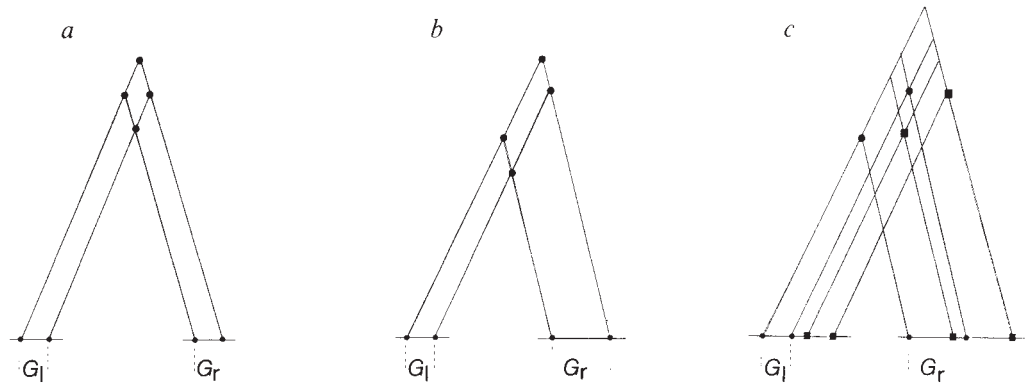


FIG. 1 Three random-dot stereograms and the results of the application of PMF to each of them. Each stereogram is constructed as follows. A 5% random dot pattern (256 units square) is replicated in the left image with a spacing between copies of G_L pixels and in the right image with spacing G_R pixels. The right-hand column shows the respective profiles through disparity space of the unique matches identified by PMF: for purposes of illustration, all matches from all over the associated stereo pair are compressed into a single cross-section, with disparity planes varying from convergent from bottom to top of the figure. For the experiments reported here, PMF's support neighbourhood was defined as a circular patch of radius 10 pixels in the left image, and the disparity range for the pool of potential matches was ± 20 pixels. In *a*, $G_L=5$ and $G_R=10$, unique matches were obtained by PMF (when run with a limiting disparity gradient of 0.5) for 5,814 of the possible 6,246 image primitives. These were distributed densely within four disparity planes so that they appear as almost solid lines in the profile figure. Psychophysical data³ for stereograms similar to *a* are similar in that some human observers report stable fusion of up to four transparent depth planes. The inner pair of dense planes (at disparities of 0 and 5 pixels)—called the 'correct solution' in the text because

they have the disparities of the two copies of the random dot texture—comprise only slightly more selected matches (1,330 and 1,226 uniquely determined matched points, respectively) than the outer pair of dense 'ghost' planes at 10 and -5 unit disparities (1,113 unique matches in each plane). This distribution reflects the marginally increased chance of matches between the correct planes exchanging support than in the ghost cases. If the disparity gradient limit used in PMF is increased to 1.0 (the psychophysical limit of fusion) qualitatively similar results are observed but the number of unique matches in the correct planes increases (1,840 and 1,716) and in the ghosts planes decreases (390 and 447). *b* ($G_L=5$ and $G_R=7$) shows that as the disparity difference between disparate copies is decreased the correct solution becomes dominant: in this case, PMF can match uniquely 5,951 matches of which most (2,351 and 2,523) exist in the inner pair of planes (at disparities of 0 and 2 pixels, respectively). *c*, Illustration of the way in which PMF selects a single surface (with 6,159 unique matches in it and a few incorrect fusions at disparities of 1 or 2 pixels from it) for the condition where $G_L=G_R=5$. This result is also in close accord with psychophysical data³.

FIG. 2 Variants of the double nail illusion. *a*, Pairs of identical objects presented to humans are generally seen as lying side by side when they originate from thin objects (such as nails, needles or pins) arranged one immediately behind the other. The 'correct' solution would require a change in the order of projection of points along matching epipolar lines in the two images⁷. *b*, Asymmetric version of the same illusion. Again the order-preserving solution is preferred. *c*, Double double nail illusion. Two sets of pairs of points in the left and right images with separations G_l and G_r between copies and with $G_r = 2G_l$. If the separation in the left image between the circular and square primitives is



less than G_l , then a change of order in the right image results.

price of less good performance on three-dimensional surfaces varying rapidly in depth.) After calculation of support scores, matches are selected according to a winner-take-all scheme in which maximally supported matches are chosen such that only one match is permitted for each left and right image feature. This final selection stage imposes the uniqueness constraint. One important feature of the algorithm is that no cost is imposed from neighbouring matches that violate the disparity gradient limit (compare with Prazdny's similar algorithm⁷). Hence PMF can deal (to an extent dependent on particular image characteristics, see below) with partially transparent surfaces that are only locally spatially cohesive^{4,7}.

Figure 1 presents examples of the kind of random dot stereograms used by Weinshall and the results of applying PMF to them. The stimuli were created by first generating a random dot pattern and then constructing each stereo image by adding another copy of it, with horizontal spacing between copies in the left and right images being termed G_l and G_r respectively. Both PMF and (some) human observers interpret stereograms like Fig. 1*a*, in which $G_r = 2G_l$, as consisting of four transparent planes of dots. When $G_r = G_l$, as in Fig. 1*c*, both PMF and humans report a single plane. Computational experiments with stimuli of this kind have revealed that the number of planes appearing in the output from PMF is dependent on a complex interaction between: (1) the disparity difference between copies in the left and right images (increasing the difference strengthens the ghost planes: for example, an intermediate condition in which $G_r = 1.4G_l$ produces two planes, Fig. 1*b*); (2) the size of the limiting disparity gradient used in the computation of local support (increasing the limit weakens the ghost planes); and (3) density of the stereogram (increasing the density strengthens the ghost planes).

Weinshall³ argued that the imposition of the uniqueness constraint for Fig. 1*a* should result in matches lying in just two disparity planes, one for each copy of the random dot pattern from which the stereo halves were made (these two planes will hereafter be called the 'correct' planes). At first sight this seems a reasonable claim because arriving at four planes suggests that each copy is being matched more than once. Yet the prediction rests on the assumption that each and every match reflecting the disparate positions of the two copies should obtain more support than competing 'incorrect' matches lying in other so-called 'ghost' planes. This assumption is false for an algorithm such as PMF, which incorporates only local support. The complex patterns of interference that arise between random dots in each of the potential transparent surfaces, correct and incorrect, are such that at least some incorrect matches will obtain more local support (and hence will be uniquely selected) than their correct counterparts.

Why do fewer planes emerge as the disparity difference decreases between copies in each image? The decrease leads to

an increase in the probability of support existing between the correct disparity planes relative to the probability of equivalent support existing between other combinations of disparity planes. The decrease also leads to a decrease in the interference between the local support structures for dot patterns in each plane. Together these factors cause the correct solutions to become increasingly dominant with decreasing disparity difference, with unique solutions tending to be consistent with the two correct surfaces for certain values of separation (see, for example, Fig. 1*b*). The limiting case is when the disparity difference is decreased to zero, which produces a single coherent surface (as in Fig. 1*c*).

Weinshall's stereograms are designed to be random-dot extensions of the double nail illusion⁸. The latter arises from just two elements in each stereo image (Fig. 2). The reported psychophysical results, and those obtained from the application of PMF, to the random dot versions, however, reveals important differences between the two cases. At the level of dot micropatterns, it is clear that when considered in isolation, each repeated dot couplet along matching rasters in the left and right random dot images forms an individual version of a stimulus pattern for generating the double nail illusion. But at the macro level of dense random dot patterns portraying transparent surfaces, complex interactions and ambiguities occur that cannot be accounted for by treating these stimuli as enlarged versions of the double nail case. For example, consider the situation illustrated in Fig. 2*c*, which shows how the ordering of dots along a pair of matching rasters fails to be preserved because of an interaction between just a pair of double dot couplets.

Contrary to Weinshall, we conclude that just because the kind of stereogram shown in Fig. 1*a* can produce the perception of four distinct depth planes, the human visual system does not necessarily *ipso facto* fail to impose the uniqueness constraint. Indeed, the phenomenon of four depth planes supports exactly the opposite conclusion, at any rate for dense textures, insofar as a stereo algorithm that imposes unique matches also produces four depth planes for equivalent stimuli.

It would be possible in principle to devise a stereo algorithm that imposed unique matches and also produced just two depth planes for Fig. 1*a* and *b*. Such an algorithm would need to incorporate a benefit for unique matches lying in the minimum number of globally cohesive planes. The human visual system does not produce the minimum number of planes consistent with unique matches and this suggests that it may use solely local neighbourhood support interactions of the kind intrinsic to the design of PMF. □

Received 7 June; accepted 24 July 1990.

1. Marr, D. & Poggio, T. *Science* **194**, 283-287 (1976).
2. Mayhew, J. E. W. & Frisby, J. P. *Artificial Intelligence* **17**, 349-386 (1981).

3. Weinshall, D. *Nature* **341**, 737–739 (1989).
4. Pollard, S. B., Mayhew, J. E. W. & Frisby, J. P. *Perception* **14**, 449–470 (1985).
5. Pollard, S. B., Porcili, J., Mayhew, J. E. W. & Frisby, J. P. *Proc. 3rd Int. Symp. Robotics Research* 19–26 (1985).
6. Burt, P. & Julesz, B. *Perception* **9**, 671–682 (1980).
7. Prazdny, S. *Biological Cybernetics* **52**, 93–99 (1985).
8. Krol, J. D. & van de Grind, W. A. *Perception* **9**, 651–669 (1980).

ACKNOWLEDGEMENTS. We thank J. Mayhew and J. Porcili for criticisms of this manuscript.

Reformation of long axon pathways in adult rat central nervous system by human forebrain neuroblasts

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THE failure of lesioned axons to regenerate over long distances in the mammalian central nervous system (CNS) is not due to an inability of central neurons to regenerate, but rather to the non-permissive nature of the CNS tissue environment^{1–4}. Regenerating CNS axons, which grow well within a peripheral nerve, for example, fail to penetrate mature CNS tissue by more than about 1 mm^{1,5,6}. Recent evidence indicates that this may be due to inhibitory membrane proteins associated with CNS oligodendrocytes and myelin^{2–4,7,8}. We report here that human telencephalic neuroblasts implanted into the excitotoxically lesioned striatum of adult rats can escape or neutralize this inhibitory influence of the adult CNS environment and extend axons along major myelinated fibre tracts for distances of up to ~20 mm. The axons were seen to elongate along the paths of the striato-nigral and cortico-spinal tracts to reach the substantia nigra, the pontine nuclei and the cervical spinal cord, which are the normal targets for the striatal and cortical projection neurons likely to be present in these implants.

Rats subjected to a unilateral ibotenic acid lesion of the caudate-putamen were injected into the same area after 7–10 days or one year later with a cell suspension prepared from the telencephalic ganglionic eminences ($n = 16$) or from the rhombencephalic lip ($n = 4$) dissected from the brains of 8–10 week-old aborted fetuses (Fig. 1). The rats were immunosuppressed to prevent rejection of the xenogeneic tissue⁹. Brains were processed for immunohistochemical analysis 12–25 weeks after implantation (Fig. 1). The human neurons and their associated axonal outgrowth were visualized using a species-specific antibody raised against a constituent of human neurofilaments (HNF) that has a molecular weight of 70,000 (refs 10, 11). Control sections from intact or ibotenic acid-lesioned rat brains were completely unstained by this antibody. This was also the case for sections from transplanted brains, identical to the ones used here but carrying implants of fetal rat or mouse ganglionic eminence tissue.

Fourteen of the 20 grafted rats (12 telencephalic and 2 rhombencephalic grafts) contained viable HNF-positive implants. Nissl-stain revealed patches of mature-looking graft neurons interspersed with areas of small, immature-looking cells. The HNF-immunoreactivity was primarily confined to densely stained fibres within the well developed areas of the implants. Adjacent sections stained with antibody against the dopamine D1 receptor-related phosphoprotein DARPP-32, used as a marker for striatal projection neurons¹², revealed DARPP-32-positive and DARPP-32-negative patches within the neuron-rich areas of the telencephalic implants. This indicates that, like

grafts of rat ganglionic eminence tissue^{13,14}, the present human telencephalic implants were composed of a mixture of striatal and non-striatal (probably, at least in part, cortical) tissue.

In seven of the brains with surviving telencephalic implants, extensive outgrowth of HNF-positive fibres occurred within the host brain (Fig. 1). The fibres could be traced in large numbers, both along the internal capsule and the cerebral peduncle caudally towards the brain stem, and along the corpus callosum and the deep layers of the cerebral cortex into large areas of the overlying cortex. The predominant fibre outgrowth was along the myelinated fibre tracts and at higher magnification (Fig. 2a and b), the HNF-positive fibres could be seen to run both within and on the surface of the individual host myelinated fibre fascicles. This long-distance fibre outgrowth also occurred in the two animals in which the striatal lesion had been made one year before the fetal cell implantation. By contrast, very little fibre outgrowth was observed from the rhombencephalic tissue implants, despite the fact that they were themselves rich in HNF-positive fibres.

The caudally directed fibres extended in slender fascicles across the caudal and medial aspects of the implant–host border, through the adjacent host striatal neuropil to join the caudally running fibre bundles of the host internal capsule (Fig. 2d). The HNF-positive fibres coursed caudally into and through the globus pallidus, where some of them branched or turned into this nucleus (Fig. 1a, and levels II and III in Fig. 1b). A few fibres deviated into the thalamus. Interesting branching patterns were seen, such as the one depicted in Fig. 2c, where the principal axon gave off a collateral branch that passed through the bundle of white matter to ramify among the cell bodies within the globus pallidus. The fibres continued as a well defined bundle in the medial part of the internal capsule, in a position similar to that of the striato-nigral and cortico-spinal pathways; along the way, axons also branched off into the subthalamic nucleus (Fig. 2e). In the region of the substantia nigra, some axons turned dorso-laterally into the overlying pars reticulata; others continued in the medial part of the cerebral peduncle (levels V–VI in Fig. 1b; and Fig. 2f) and could be traced in high numbers into the pontine nuclei at a distance of 8–10 mm from the implant. Some fibres were also identified in deep parts of the tectum. In the cerebral peduncle, the HNF-positive fibre bundles occupied a position similar to that of cortico-fugal axons labelled anterogradely from the fronto-parietal cortex in intact animals^{15,16}. In two of the animals, where the lower brain stem and upper spinal cord were included in the analysis, HNF-positive fibres were observed in the medulla oblongata at the level of the pyramidal crossing, and in the white and grey matter of the upper cervical spinal cord at a distance ~20 mm from the implant.

The retrograde fluorescent tracers Fluoro-Gold and rhodamine-labelled latex beads were injected into the globus pallidus and substantia nigra, respectively, in two rats with telencephalic implants (Fig. 1). Clusters of Fluoro-gold-positive cell bodies and scattered cells labelled with rhodamine-latex beads were identified within the mature-looking regions of the implants, thus confirming that neurons in the implants projected to these sites.

The fibres projecting into the cortex extended in large numbers along the corpus callosum, the external capsule and the forceps minor, and within the deep layers of the cortex bilaterally. Labelled fibres were distributed in the frontal, parietal and occipital cortices on both sides. Ventrally, HNF-positive fibres could be traced into the amygdaloid-piriform area at about 8–10 mm away from the implants.

In line with the idea that the formation of long fibre tracts in the CNS reflects an interaction and balance between growth-promoting and growth-inhibiting factors along the growth trajectory, it has been proposed^{2,4,7} that the limited regrowth of axotomized neurons in the adult brain or spinal cord is, at least in part, due to an active inhibition of axonal elongation exerted

by the oligodendrocytes and myelin components of mature white matter, and that blockade of this inhibition can markedly improve axonal regeneration⁴. At least certain types of immature CNS neurons may be relatively insensitive to these growth-inhibiting factors during a limited period of development. This period may coincide with the time of active axonal elongation and pathway formation when growing axons are known to have special growth substrate requirements^{17,18}. The human neuroblasts, which have a very protracted development, are likely to remain in their phase of active axonal elongation for a long period after implantation, thus making it possible for them to extend axons over long distances in the lesioned host brain. Mouse telencephalic neuroblasts, with a shorter developmental time span, show more restricted axon outgrowth (as visualized with a mouse-specific antibody) when grafted into the rat striatum under identical (immunosuppressed) conditions (K.W. *et al.*, submitted). Indeed, with implants of fetal rodent neurons, extensive axonal projections in lesioned adult brains have previously been observed only when the implants are placed close

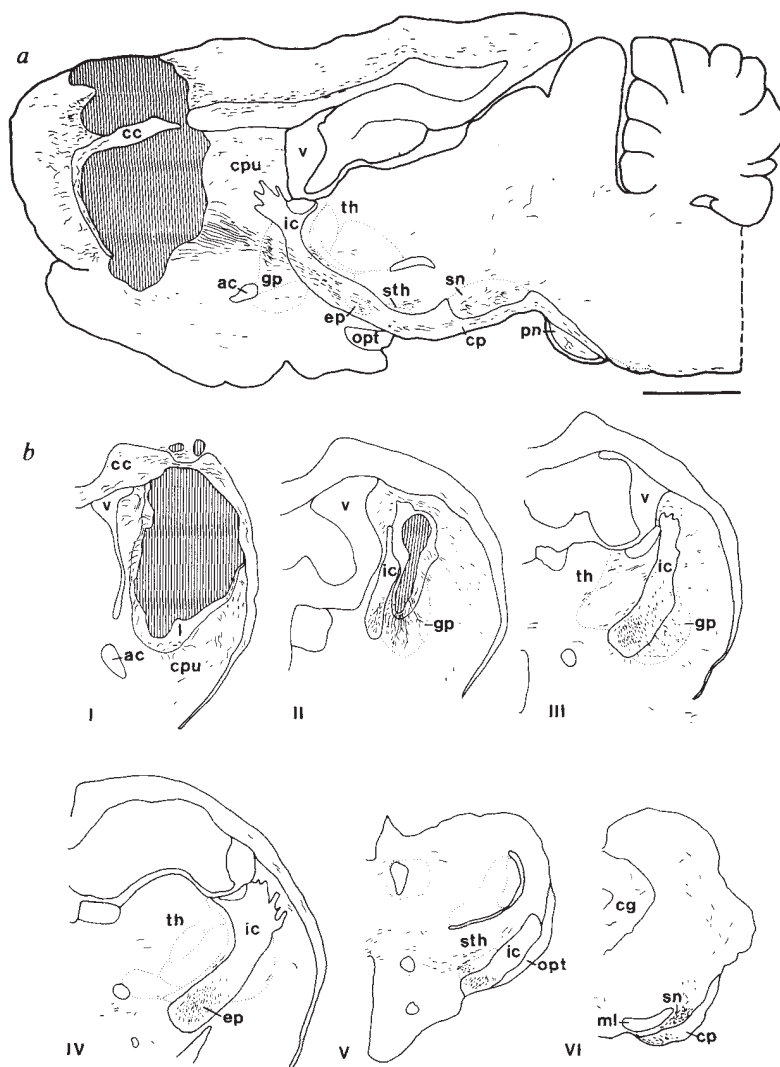
(generally <1 mm) to the target¹⁹⁻²⁶. This difference in growth capacity may also reflect the large difference in size between rodent and human neurons: the length of, for example, the striato-nigral projection in the human brain (~30 mm) is ~10 times longer than that of the mouse. The considerably larger dimensions of the pathways normally formed by the human telencephalic neuroblasts may thus be expressed as a greater growth capacity in the implant situation as well.

Axonal growth from the telencephalic grafts showed a remarkable selectivity, and it was specific in the sense that it did not occur from identically positioned rhombencephalic neuroblasts. The descending HNF-positive axons formed a well defined bundle at a position closely matching that of the striato-nigral and cortico-ponto-spinal pathways in the intact brain and they branched off into nuclei normally densely innervated by these pathways.

These results indicate that the basic requirements for the reformation of long axonal pathways may be present in the lesioned adult mammalian CNS, provided that the regrowing

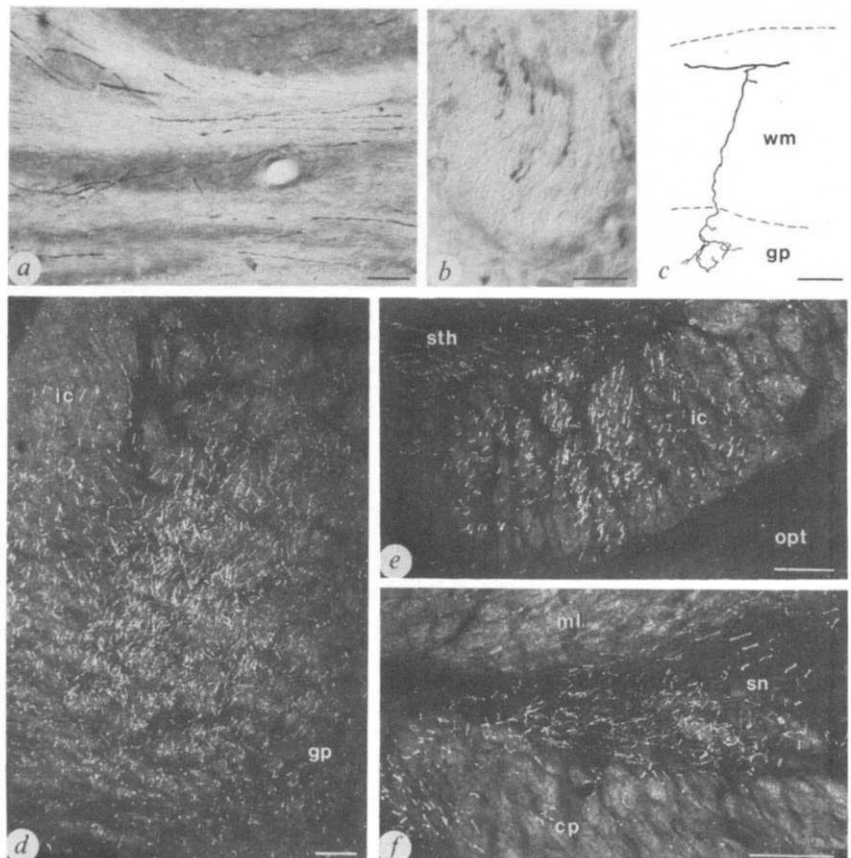
FIG. 1 Semischematic drawings of HNF-stained sections from adult rat brains with intrastriatal implants of human fetal ganglionic eminence tissue, showing the extent of the HNF-positive efferent projections. *a*, Drawing of 3-4 superimposed sagittal sections from a rat with a 23-week-old graft (hatched area). *b*, Coronal sections from 6 different rostral-to-caudal levels, 21 weeks after implantation. ac, Anterior commissure; cpu, intact caudate-putamen; cc, corpus callosum; cp, cerebral peduncle; ep, entopeduncular nucleus; gp, globus pallidus; ic, internal capsule; l, lesioned host caudate-putamen; opt, optic tract; pn, pontine nuclei; sth, subthalamic nucleus; sn, substantia nigra; th, thalamus; v, lateral ventricle. Scale bars, 2 mm.

METHODS. A total of 20 adult female Sprague-Dawley rats (200 g body weight) were anaesthetized with Equithesin (0.3 ml 100 g⁻¹), placed in a Kopf stereotaxic frame, and unilaterally injected into the head of the caudate-putamen with 14 µg ibotenic acid (Sigma; 10 µg µl⁻¹) as previously described²⁵. One to two weeks later the rats received injections into the lesioned area of cell suspensions prepared either from the ganglionic eminences (*n*=14) or the rhombencephalic lip (*n*=4), dissected from the brains of aborted human fetuses of 8-10 weeks postmenstrual age. Two additional animals received implants of ganglionic eminence tissue at 1 yr after the ibotenic acid lesion. With the approval of the Research Ethical Committee at the University of Lund, tissue was retrieved from the fragmented material of 8 fetuses from suction abortions. Cell suspensions were prepared as before²⁷. In brief, the dissected pieces were collected in 0.6% glucose-saline, incubated with 0.1% trypsin (20 min, 37 °C), rinsed and triturated into a milky cell suspension through the tip of a fire-polished Pasteur pipette to a total volume of 50-100 µl for each dissected region. Between 6 and 9 µl suspension, containing ~2-4 × 10⁵ viable cells, were injected into each of the rats, and divided over 2 or 3 injection sites. From the day of implantation, rats were immunosuppressed by daily injections of cyclosporin A (Sandoz; 10 mg kg⁻¹, i.p.)⁹. Despite this immunosuppressive treatment, about one third (6 out of 20; 4 with telencephalic, and 2 with rhombencephalic grafts) of the grafts were rejected. At 12-25 weeks after transplantation, the rats were deeply anaesthetized with chloral hydrate (350 mg kg⁻¹), and perfused through the ascending aorta with 50 ml saline at room temperature, followed by 300 ml ice-cold phosphate-buffered 4% paraformaldehyde. After 2 h of postfixation and overnight incubation in 20% sucrose, brains were cut on a freezing microtome (30 µm) in the sagittal or coronal planes. Sections were incubated for 1 h with 10% normal horse serum in 0.02 M potassium phosphate-buffered saline, followed by a 48-h incubation with a mouse primary antiserum specifically recognizing the 70K polypeptide (molecular weight, 70,000) constituent of human neurofilaments (Serotec, UK) at a dilution of 1:200 in potassium phosphate-buffered saline with 2% normal horse serum, and 0.3% Triton-X100. To test the specificity of the human neurofilament antiserum, control sections from intact or lesion-only rats, and from rats with fetal rat or mouse implants, were processed according to the same protocol. An avidin-biotin-peroxidase complex system (Vector)



was used to detect the primary antiserum, with 3,3'-diaminobenzidine as chromogen, and osmium-intensification of the reaction product. Alternate sections were incubated with a primary antiserum raised (in mouse) against DARPP-32 at a dilution of 1:20,000, and reacted according to the same protocol, except that no Triton-X100 was used. Two of the rats with telencephalic grafts received injections from micropipettes of the retrograde tracers Fluoro-Gold (Fluorochrome Inc.) and rhodamine-labelled latex beads (Lumafuor Inc.) into the host brain globus pallidus and substantia nigra, respectively, at 1-2 week before death²⁵.

FIG. 2 Details of the HNF-immunoreactive efferent projections extending into the host brain from implants of ganglionic eminence tissue. *a* and *b*, High-power brightfield micrographs from sagittal and coronal sections, respectively, showing the position of the efferent projecting HNF-immunoreactive fibres along and inside the white matter bundles of the host internal capsule. *c*, High-magnification camera lucida drawing from a sagittal section illustrating how a thin axonal branch extended from a thicker main axon running within a white matter (wm) bundle, into the adjacent globus pallidus (gp), where it ramified among the host cell bodies. This branching pattern was often found in this region in the animals with telencephalic implants, and also in the rats with 1-year-old lesions at the time of implantation, as illustrated here. *d–f*, Darkfield micrographs from coronal sections corresponding to levels III, V, and VI, respectively, of the drawings in Fig. 1*b*. *d*, Section from the level of the rostral globus pallidus (gp) showing large numbers of HNF-positive efferents from the graft extending along and inside the host internal capsule (ic) bundles. At this level, some fibres were seen to branch off into the gp. *e*, The fibres continued caudally in the medial part of the internal capsule (ic), with some fibres running off into the region of the subthalamic nucleus (sth). *f*, Further caudally, some HNF efferents extended into the substantia nigra (sn), and others continued in the medial part of the cerebral peduncle (cp) to reach even more caudal host brain regions. Scale bars: *a*, 50 μ m; *b* and *c*, 25 μ m; *d–f*, 200 μ m.



axonal elements can escape or neutralize the inhibitory features of the growth trajectory. Extensive axonal outgrowth from implanted fetal neurons in the adult brain depends on the presence of a preceding lesion^{23,26}. However, the observation that the long fibre outgrowth was also present when the telencephalic cells were implanted one year after the striatal lesion indicates that the necessary guidance and growth-promoting

mechanisms persist in the chronically lesioned CNS as well. The extensive pathway-forming capacity of the slowly developing human neuroblasts should provide new possibilities for the analysis of the cellular interactions underlying successful regeneration in the CNS. □

Received 3 April; accepted 9 August 1990.

- David, S. & Aguayo, A. J. *Science* **214**, 931–933 (1981).
- Schwab, M. E. & Thoenen, H. *J. Neurosci.* **5**, 2415–2423 (1985).
- Carbonetto, S., Evans, D. & Cochard, P. *J. Neurosci.* **7**, 610–620 (1987).
- Schnell, L. & Schwab, M. E. *Nature* **343**, 269–272 (1990).
- Ramon y Cajal, S. *Degeneration and Regeneration of the Nervous System* (Hafner, New York, 1959).
- Vidal-Sanz, M., Bray, G. M., Villegas-Perez, M. P., Thanos, S. & Aguayo, A. J. *J. Neurosci.* **7**, 2894–2909 (1987).
- Schwab, M. E. & Caroni, P. *J. Neurosci.* **8**, 2381–2393 (1988).
- Crutcher, K. A. *Expl. Neurol.* **104**, 39–54 (1989).
- Brundin, P., Nilsson, O. G., Gage, F. H. & Björklund, A. *Expl. Brain Res.* **60**, 204–208 (1985).
- Alfonso, F., Darmon, M., Forest, N. & Paulin, D. in *The Role of Cell Interactions in Early Neurogenesis* (eds Duprat, A. M., Kato, A. C. & Weber, M.) 157–176 (Plenum, New York, 1984).
- Julien, J. P., Tretjakoff, I., Beaudet, L. & Peterson, A. *Genes Dev.* **1**, 1085–1095 (1987).
- Ouimet, C. C., Miller, P. E., Hemmings Jr, H. C., Walaas, I. & Greengard, P. *J. Neurosci.* **4**, 111–124 (1984).
- Victorin, K., Ouimet, C. C. & Björklund, A. *Eur. J. Neurosci.* **1**, 690–701 (1989).
- Graybiel, A. M., Liu, F.-C. & Dunnett, S. B. *J. Neurosci.* **9**, 3250–3272 (1989).
- Doucet, G. et al. *Expl. Neurol.* **106**, 1–19 (1989).
- Sesack, S. R., Deutch, A. Y., Roth, R. H. & Bunney, B. S. *J. comp. Neurol.* **290**, 213–242 (1989).
- Cohen, J., Burne, J. F., Winter, J. & Barlett, P. *Nature* **322**, 465–467 (1986).
- Tomaselli, K. J., Neugebauer, K. M., Bixby, J. L., Lilien, J. & Reichardt, L. F. *Neuron* **1**, 33–43 (1988).
- Björklund, A., Stenevi, U. & Svendgaard, N. A. *Nature* **262**, 787–790 (1976).
- Björklund, A., Stenevi, U., Schmidt, D., Dunnett, S. B. & Gage, F. H. *Acta Physiol. Scand. Suppl.* **522**, 9–18 (1983).
- Nornes, H., Björklund, A. & Stenevi, U. *Cell Tiss. Res.* **230**, 15–35 (1983).
- Foster, G. A. et al. *Expl. Brain Res.* **60**, 427–444 (1985).
- McLoom, L. K., McLoom, S. C., Chang, F.-L.F., Steedman, J. G. & Lund, R. D. in *Neural Grafting in the Mammalian CNS* (eds Björklund, A. & Stenevi, U.) 267–283 (Elsevier, Amsterdam, 1985).
- Sotelo, C. & Alvarado-Mallart, R. M. *Neuroscience* **20**, 1–22 (1987).
- Victorin, K., Simerly, R. B., Isaacson, O., Swanson, L. W. & Björklund, A. *Neuroscience* **30**, 313–330 (1989).
- Zhou, C.-F. & Raisman, G. *Neuroscience* **32**, 349–362 (1989).
- Björklund, A., Stenevi, U., Schmidt, R. H., Dunnett, S. B. & Gage, F. H. *Acta Physiol. Scand. Suppl.* **522**, 1–75 (1983).

ACKNOWLEDGEMENTS. We thank A. Flasch, U. Jarl and G. Stridsberg for technical assistance, and Dr Greengard for DARPP-32 antibody. This study was supported by the Swedish MRC and the Bank of Sweden Tricentenary Fund.

The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor α gene to a novel transcribed locus

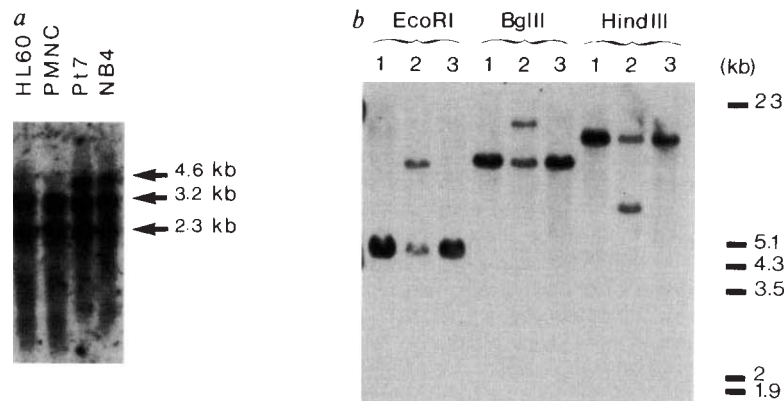
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RETINOIC acid is a vitamin A derivative with striking effects on development and cell differentiation^{1–3}. Several nuclear retinoic acid receptors (RARs), acting as ligand-inducible transcription factors, have been characterized^{4–8} and indirect evidence suggests that they have distinct roles^{9–11}. One of the most intriguing properties of retinoic acid is its ability to induce *in vivo* differentiation of acute promyelocytic leukaemia (APL) cells into mature granulocytes, leading to morphological complete remissions^{12–13}. Because the *RAR α* gene maps to chromosome 17q21 (ref. 14), close to the t(15;17) (q21–q11–22) translocation specifically associated with APL¹⁵, we analysed *RAR α* gene structure and

FIG. 1 *a*, Retinoic acid receptor α gene expression in patient 7 and in the NB4 APL cell line. Northern blot analysis was performed with an *RAR α* cDNA probe⁵ using 5 μ g of poly(A)⁺ RNAs extracted from the human myeloid leukaemic cell line HL60, human peripheral blood polymorphonuclear cells (PMNC), fresh leukaemic cells from patient 7 (Pt7) and the NB4 cell line derived from blast cells of patient 7. *b*, Rearrangement of the *RAR α* gene in NB4 cells. Genomic DNAs from NB4 (lane 2) as well as from two controls, PMNC (lane 1) and HL60 (lane 3), were digested with the indicated restriction enzymes. The Southern blot was hybridized to an *RAR α* cDNA probe specific for the second exon (V. Giguère, personal communication) which encodes the B and C1 domains²⁴. The 147-bp fragment used as a probe was synthesized using PCR²⁵ and two 16-mer oligonucleotides (positions 406 and 553 in ref. 5) as primers. The DNA markers are aligned.



expression in APL cells. We report here that, in one APL-derived cell line, the *RAR α* gene has been translocated to a locus, *myl*, on chromosome 15, resulting in the synthesis of a *myl/RAR α* fusion messenger RNA. Using two probes located on either side of the cloned breakpoint, we have found genomic rearrangements of one or other locus in six patients out of eight, demonstrating that the *RAR α* and/or *myl* genes are frequently rearranged in APL and the breakpoints are clustered. These findings strongly implicate retinoic acid receptor α in leukaemogenesis.

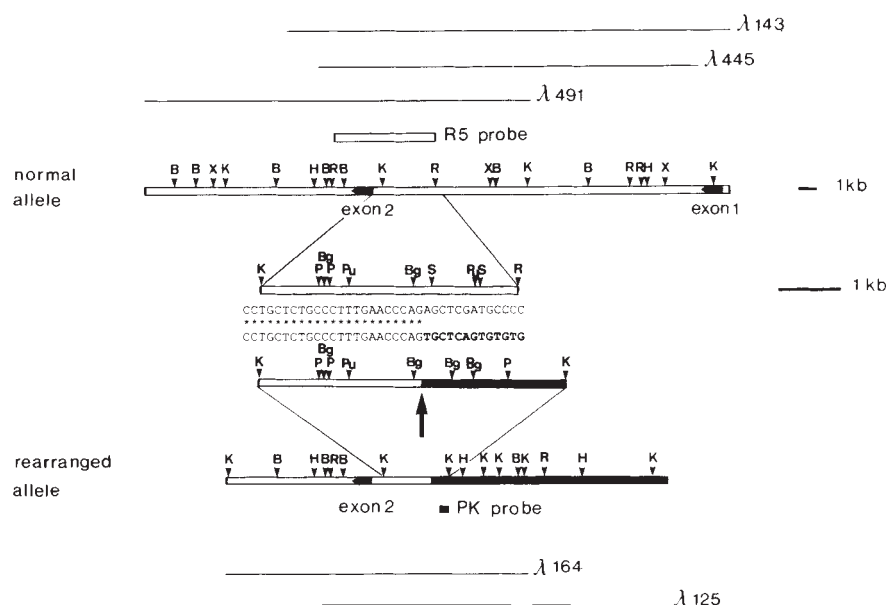
Preliminary studies suggested that the *RAR α* gene was rearranged and its expression altered in APL patients (C.C. and P. Ballerini, personal communication). To establish this, bone marrow cells were collected from a t(15;17)-positive APL patient (Pt7) and a stable promyelocytic cell line (NB4) was derived from a primary culture of the blast cells (M.L. *et al.*, submitted). Expression studies in the fresh leukaemic cells and the cell line revealed an abnormal 4.6-kilobase (kb) *RAR α* mRNA in both (Fig. 1*a*). Southern blot analysis revealed an additional *Eco*RI, *Bgl*II and *Hind*III restriction fragment in the DNA of Pt7 and NB4, compared with nonleukaemic cells, indicating that the *RAR α* gene was identically rearranged in both samples (not shown). Using several subregions of the *RAR α* complementary DNA as probes, we demonstrated that a short 147-base pair (bp) DNA fragment, corresponding to exon 2 (V. Giguère, personal communication), detected the DNA rearrangement (Fig. 1*b*) whereas the other probes did not.

A genomic library was generated from NB4 DNA and hybrid-

ized to the radiolabelled probe for *RAR α* exon 2. Three clones (λ 143, λ 445, λ 491) correspond to 28 kb of germline DNA, as shown by the sizes of hybridizing restriction fragment (Fig. 2). On the genomic map, the presence of the first and second α exons defines the direction of transcription. Two other overlapping clones (λ 125, λ 164) cover 21 kb of DNA, of which only 10 kb share a common restriction map with the germline *RAR α* gene (Fig. 2), suggesting that these two λ phages originate from the rearranged allele. Nucleotide sequence analysis of both alleles reveals that the breakpoint on chromosome 17 is 103 bp from the common *Bgl*II site (Fig. 2). The PK probe, originating from the transposed sequences present in clone λ 125 (Fig. 2), was hybridized *in situ* and localized to the q23-q24 bands of chromosome 15 (data not shown), establishing that the cloned rearranged allele contains the t(15;17) translocation breakpoint. Altogether, these data indicate that, in this patient, the promoter region and the first exon of the *RAR α* gene have been replaced by a DNA fragment originating from chromosome 15 as a consequence of the translocation.

To determine whether the same type of genomic alteration was encountered in other t(15;17)-positive APL patients, leukaemic DNA samples from eight subjects¹³ were analysed by Southern blotting. Hybridization of the *Eco*RI-restricted DNA to the R5 genomic probe, specific for chromosome 17 (Fig. 2), revealed a rearrangement in three out of eight patients, but not in the normal or non-APL leukaemic controls (Fig. 3*a*). When these DNA digests were rehybridized to the PK probe,

FIG. 2 Comparative restriction maps of the normal and rearranged loci. Partial *Mbo*I digests of genomic DNA from NB4 cells were cloned into the λ vector EMBL 3 using standard procedures. The library was screened with a retinoic acid receptor α cDNA probe specific for exon 2 (see Fig. 1*b*). Three (λ 143, λ 445, λ 491) and two (λ 164, λ 125) positive overlapping clones corresponding respectively to 27 kb of germline and 21 kb of rearranged alleles were analysed. Open bar, sequences from chromosome 17; solid bar, sequences from chromosome 15. The presence of the retinoic acid receptor α exons 1 and 2 are indicated by horizontal arrows. A detailed restriction map of the precise breakpoint (indicated by a vertical arrow) and the corresponding unrearranged locus are shown as well as the partial nucleotide sequence of the two loci. The 300-bp *Bgl*II-*Sma*I and the 450-bp *Bgl*II fragments corresponding respectively to the germ-line and to the translocated alleles encompassing the precise breakpoint region were cloned into M13 vectors and sequenced. The 5-kb *Eco*RI fragment derived from chromosome 17 in the normal allele (R5 probe) and the 0.6-kb *Pst*I-*Kpn*I fragment specific for chromosome 15 in the rearranged locus (PK probe) are indicated. Restriction sites are: R, *Eco*RI; B, *Bam*HI; H, *Hind*III; X, *Xho*I; K, *Kpn*I; P, *Pst*I; Bg, *Bgl*II; Pu, *Pvu*II; S, *Sma*I.



derived from chromosome 15, four out of seven APL patients exhibited a DNA rearrangement (Fig. 3b). A germline hybridization pattern with both probes was found in two patients only. The data are summarized in Fig. 3c. For patient 9, the extra band for both probes comigrates (Fig. 3a and b), strongly suggesting that this restriction fragment contains the t(15;17) breakpoint. These results indicate that both loci are frequently rearranged in APL. Assuming that these genomic alterations correspond to the t(15;17) translocations, the data show that the chromosomal breakpoints are tightly clustered.

As the t(15;17) translocation in NB4 deletes the promoter sequences in the rearranged *RARα* gene, we speculated that the chromosome 15-derived region might contain a gene that could control *RARα* transcription. When the PK probe was hybridized to *TaqI*-restricted DNA from yeast, cow, mouse, chicken and human under conditions of relaxed stringency, specific bands appeared in the DNA of vertebrates but not of yeast, suggesting that this probe contains an exon (data not shown). To search for potential transcripts, a PK-M13-derived single-stranded probe (Fig. 4a) was hybridized to poly(A)⁺ mRNA purified from several human haematopoietic cell lines. Five major mRNA species, ranging from 1.8 to 12 kb, were detected (Fig. 4a), demonstrating the presence of an exon in the PK probe. It should be noted that this locus, for which we propose the name *myl* for myelocytic leukaemia, is transcribed in the same direction as *RARα* on the translocated allele. To localize the exon, the PK probe was sequenced and computer analysis (PREDICTOR program, ref. 16) showed that a 144-bp region, flanked by canonical splice acceptor and donor sequences, had a very high probability of being an exon (data not shown). Comparison with a protein data bank failed to demonstrate any significant similarity, suggesting that *myl* is a previously undescribed gene.

When *myl* gene expression was studied in NB4 cells, an abundant abnormal transcript of 4.6 kb (Fig. 4a), which comigrates with the abnormal *RARα* mRNA (Fig. 1a), was detected, suggesting the presence of a cotranscript between the two genes. The 4.6-kb transcript was not present in non-APL leukaemic cells. To substantiate the hypothesis that the t(15;17) translocation generates a *myl/RARα* chimaeric transcript, we performed a reverse transcription/polymerase chain reaction on NB4 and HL60 mRNAs, using primers derived from *RARα* exon 2 and from the 144-bp exon of *myl* (primers 01, 02 and 03 in Fig. 4b).

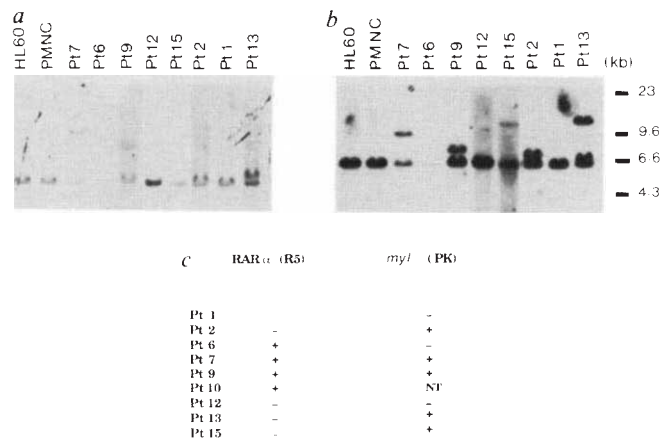


FIG. 3 Genomic abnormalities in APL patients. a and b, Control human DNAs from HL60 and PMNC were run next to genomic DNA of patient 7 (Pt7) and 7 other APL patients (Pts 1, 2, 6, 9, 12, 13, 15) harbouring the specific t(15;17) translocation. DNAs were digested by *EcoRI* and hybridized to the chromosome 17-derived R5 probe (a). The filters were subsequently rehybridized to the chromosome 15-derived PK probe (b). c, DNA rearrangements detected in APL patients with either the R5 or PK probe, corresponding to the retinoic acid receptor α (*RARα*) and *myl* loci respectively. The presence (+) or absence (-), of genomic alterations are summarized. NT, not tested.

When the amplification products were analysed by gel electrophoresis, a sharp band of ~450 bp was observed in NB4 but not in the HL60 negative control. This band hybridized by Southern blotting to the *myl*- and *RARα*-derived 04 and 05 probes respectively (Fig. 4c), indicating that the *myl/RARα* junction was amplified in this experiment. These data establish that, in the NB4 cell line, the t(15;17) translocation generates a *myl/RARα* chimaeric transcript.

It has been suggested that altered nuclear receptors may be implicated in oncogenesis¹⁷. The oncogenic derivative of the thyroid-hormone receptor, *v-erbA*, for example, acts as a constitutive repressor of thyroid hormone-responsive genes¹⁸⁻¹⁹. On this basis, it was proposed that the *v-erbA* product competes with the wild-type receptor for binding to hormone-responsive elements and exerts its transforming effects by blocking thyroid-hormone-mediated erythrocytic differentiation.

As retinoic acid exerts profound effects on differentiation and proliferation of both non-neoplastic and malignant cells¹⁻², alteration of retinoic acid receptors might be involved in oncogenesis. The *RARβ* gene has been shown to be rearranged in a human hepatocellular carcinoma as a result of insertion of hepatitis B virus sequences. Expression of the fusion gene was not documented, however²⁰. Our demonstration of a frequent

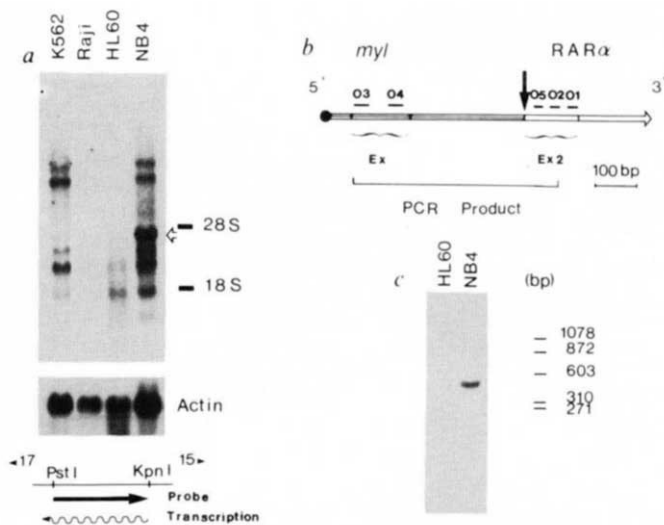


FIG. 4 Analysis of *myl* transcription. a, Expression study of the *myl* locus. Poly(A)⁺ RNA from different human haematopoietic cell lines (K562, Raji, HL60) and from the APL-derived cell line NB4 was blotted onto a nylon filter in 0.05 N NaOH and hybridized to the PK single-stranded DNA probe. The 600-bp *PstI*-*KpnI* fragment derived from chromosome 15 was subcloned into M13mp18 to generate a high specific activity (>10⁹ c.p.m./μg⁻¹) single-stranded probe. The orientation of the single-stranded probe is indicated as well as the deduced sense of transcription. The 4.6-kb abnormal *myl* transcript in NB4 RNA is indicated by an open arrow. The filters were subsequently hybridized to a β -actin probe as a quantitative control. b, Schematic description of the reverse transcriptase/polymerase chain reaction (PCR) approach which was performed as described in ref. 26. Five 20-mer oligonucleotides were synthesized for priming and hybridization: O1, O2 and O5 located within retinoic acid receptor α (*RARα*) exon 2 (Ex2) complementary to the + strand at positions 553, 443 and 406 respectively⁵, O3 and O4 within the presumed exon of the *myl* locus (Ex) positioned respectively 72 and 161 bp downstream from the *KpnI* site. Poly(A)⁺ mRNA (1 μg) from NB4 or HL60 cells was reverse transcribed using O1. The cDNA was then subjected to PCR after addition of O2 and O3 as primers. The open bar indicates sequences from chromosome 17-*RARα* locus and the horizontal striped-bar represents sequences from the chromosome 15-*myl* locus. The sense of transcription is denoted 5' to 3'. The arrow corresponds to the presumed fusion point between the two transcripts. c, Southern blot analysis of the amplification products obtained after PCR from cDNAs for NB4 and HL60 RNAs. Probes were oligonucleotides O4 or O5 radiolabelled at the 5' end using [³²P]ATP and T4 kinase, to a specific activity of 8 × 10⁶ c.p.m./pmol⁻¹. Both experiments gave similar results and one hybridization only is shown. The DNA markers are aligned.

involvement of the *RAR α* gene in APL provides the first direct link between an alteration of a nuclear receptor and a specific human cancer. One can hypothesize that such rearrangement led to the production of a retinoic acid receptor α whose N-terminal region was deleted and substituted by *myl*-derived sequences. It has been shown that the corresponding domain of the steroid hormone receptors may be involved in both transactivation and target gene specificity²¹⁻²³. The availability of a retinoic acid responsive element¹¹ should allow assessment

of the transactivating properties of the *myl/RAR α* fusion protein. In particular, it will be possible to determine whether or not the altered retinoic acid receptor behaves as a dominant negative mutant that might block the expression of retinoic acid target genes presumably involved in granulocytic differentiation.

In this context, it will be most interesting to clarify the apparent paradox between the differentiating effect of retinoic acid in APL and the proposed leukaemogenic role of the α retinoic acid receptor in this disease. □

Received 6 July; accepted 11 September 1990.

1. Roberts, A. & Sporn, M. In *The Retinoids* (eds Sporn, M., Roberts, A. & Goodman, D.) 209-286 (Academic Press, Orlando, 1984).
2. Strickland, S. & Mahdavi, M. *Cell* **15**, 393-403 (1978).
3. Thaller, C. & Eichele, G. *Nature* **327**, 625-628 (1987).
4. Giguere, V., Ong, E. S., Segui, P. & Evans, R. M. *Nature* **330**, 624-629 (1987).
5. Petkovich, M., Brand, N. J., Krust, A. & Chambon, P. *Nature* **330**, 444-450 (1987).
6. de Thè, H., Marchio, A., Tiollais, P. & Dejean, A. *Nature* **330**, 667-670 (1987).
7. Brand, N. *et al.* *Nature* **332**, 850-853 (1988).
8. Zelent, A., Krust, A., Petkovich, M., Kastner, P. & Chambon, P. *Nature* **339**, 714-717 (1989).
9. de Thè, H., Marchio, A., Tiollais, P. & Dejean, A. *EMBO J.* **8**, 429-433 (1989).
10. Dollé, P. *et al.* *Nature* **342**, 702-705 (1989).
11. de Thè, H., Vivanco-Ruiz, M. D. M., Tiollais, P., Stunnenberg, H. & Dejean, A. *Nature* **343**, 177-180 (1990).
12. Huang, M. E. *et al.* *Blood* **72**, 567-571 (1988).
13. Castaigne, S. *et al.* *Blood* (in the press).
14. Mattei, M. G., Petkovich, M., Mattei, J. F., Brand, N. & Chambon, P. *Hum. Genet.* **80**, 186-187 (1988).
15. Larson, R. A. *et al.* *Am. J. Med.* **76**, 827-841 (1984).
16. Claverie, J. M. & Bouguerel, L. *Nucleic Acids Res.* **14**, 179-196 (1986).

17. Green, S. & Chambon, P. *Nature* **324**, 615-617 (1986).
18. Sap, J., Munoz, A., Schmitt, J., Stunnenberg, H. & Vennart, B. *Nature* **340**, 242-244 (1989).
19. Damm, K., Thompson, C. & Evans, R. *Nature* **339**, 593-597 (1989).
20. Dejean, A., Bouguerel, L., Grzeschik, K. H. & Tiollais, P. *Nature* **322**, 70-72 (1986).
21. Hollenberg, S., Guiguere, V., Segui, P. & Evans, R. *Cell* **49**, 39-46 (1987).
22. Kumar, V. *et al.* *Cell* **51**, 941-951 (1987).
23. Tora, L., Gronemeyer, H., Turcotte, B., Gaub, M. P. & Chambon, P. *Nature* **333**, 185-188 (1988).
24. Green, S., Kumar, V., Theulaz, I., Wahli, W. & Chambon, P. *EMBO J.* **7**, 3037-3044 (1988).
25. Saiki, R. *et al.* *Science* **239**, 487-491 (1988).
26. Kawasaki, E. S. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **85**, 5698-5702 (1988).

ACKNOWLEDGEMENTS. The pSG5 retinoic acid receptor α plasmid was a gift from P. Chambon. We thank P. Tiollais and J. Weissenbach for support and discussions; M. G. Mattei for *in situ* hybridization; R. Berger, P. Ballerini and S. Gisselbrecht for their help; L. Bouguerel for computer analysis; A. Marchio and N. Balitrand for technical assistance; and D. Holmes for revising the manuscript. We also thank the physicians participating in the RA therapy trial for referring the patients. Requests for the cell line and biomaterials derived from this cell line to be addressed to M.L. This work was supported by grants from the Fondation pour la Recherche Médicale, the Commission of the European Communities, the Ligue Nationale Française contre le Cancer, and the Association pour la Recherche contre le Cancer.

An essential role for a phospholipid transfer protein in yeast Golgi function

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PROGRESSION of proteins through the secretory pathway of eukaryotic cells involves a continuous rearrangement of macromolecular structures made up of proteins and phospholipids. The protein SEC14p is essential for transport of proteins from the yeast Golgi complex¹. Independent characterization of the *SEC14* gene² and the *PIT1* gene³, which encodes a phosphatidylinositol/phosphatidylcholine transfer protein in yeast, indicated that these two genes are identical. Phospholipid transfer proteins are a class of cytosolic proteins that are ubiquitous among eukaryotic cells and are distinguished by their ability to catalyse the exchange of phospholipids between membranes *in vitro*⁴. We show here that the *SEC14* and *PIT1* genes are indeed identical and that the growth phenotype of a *sec14-1^{ts}* mutant extends to the inability of its transfer protein to effect phospholipid transfer *in vitro*^{4,5}. These results therefore establish for the first time an *in vivo* function for a phospholipid transfer protein, namely a role in the compartment-specific stimulation of protein secretion.

The phosphatidylinositol/phosphatidylcholine (PtdIns/PtdCh) transfer protein from *Saccharomyces cerevisiae* has been purified and partial amino-acid and nucleotide sequences for the protein and the 5' end of its corresponding structural gene (*PIT1*), respectively, have been determined³. We noted that the N-terminal 30 residues of the purified PtdIns transfer protein were identical to the 30 residues of the SEC14p (ref. 2) that were inferred to follow the initiator methionine on the basis of nucleotide sequence data. Moreover, the essential *PIT1* gene had an intron with the same position and sequence as the *SEC14* intron, and encoded a cytosolic polypeptide of relative molecular mass 35,000. These data strongly suggested that

SEC14 was allelic to *PIT1*.

The identity between SEC14p and the PtdIns transfer protein was unambiguously confirmed by a combination of genetic and molecular criteria. These experiments were facilitated by the isolation of *sec14^{ts}* mutants¹ which have been characterized as the conversion of residue 266 of the SEC14p primary translation product from Gly to Asp (ref. 6). Genetic evidence in support of the *SEC14/PIT1* allelism was obtained by complementation and nucleotide sequence analysis. Strain CTY1-1A (*MATa*, *ura3-52*, *sec14-1^{ts}*) was transformed with a yeast *PIT1* centromeric plasmid carrying *URA3* as a selectable marker. Transformants to Ura⁺ prototrophy were selected and tested for growth at 37 °C, a restrictive temperature for *sec14^{ts}* mutants. The *PIT1* gene in single copy restored wild-type growth to strain CTY1-1A. Thus, SEC14p and the PtdIns transfer protein were functionally identical. We confirmed that they were the same protein by completing the *PIT1* nucleotide sequence and showing that it was identical to the *SEC14* gene (not shown). This allelism of *SEC14* and *PIT1* established that *SEC14* is the structural gene for the yeast PtdIns transfer protein.

Our demonstration that *SEC14* is the structural gene for the yeast transfer protein suggested that the primary biochemical defect in *sec14^{ts}* mutants might be the inability of these cells to effect proper mobilization of PtdIns and/or PtdCh under restrictive conditions. To test this prediction directly, cytosolic fractions from isogenic *SEC14* and *sec14-1^{ts}* strains were assayed for both PtdIns and PtdCh transfer activity *in vitro*. The cytosolic fractions from both strains had comparable PtdIns transfer activity when assayed at the permissive temperature of 25 °C (Fig. 1a). Although short preincubation of the extracts at 37 °C followed by assay for PtdIns transfer at that same restrictive temperature did not significantly affect the transfer protein activity of the *SEC14* strain, activity in the extract from the *sec14-1^{ts}* strain was almost completely abolished under these conditions (Fig. 1b). Moreover, this transfer protein defect was completely reversed when a *CEN4-PIT1* plasmid was introduced into the *sec14-1^{ts}* strain (not shown). We also noted that, in agreement with the reversibility of the *sec14-1^{ts}* defect *in vivo*¹, loss of PtdIns transfer activity in extracts from the *sec14-1^{ts}* strain preincubated and assayed at 37 °C was partially restored when assayed at 25 °C (Fig. 1c). Also, extracts prepared from the *sec14-1^{ts}* strain grown for several hours at the restrictive temperature contained significant transfer protein activity when

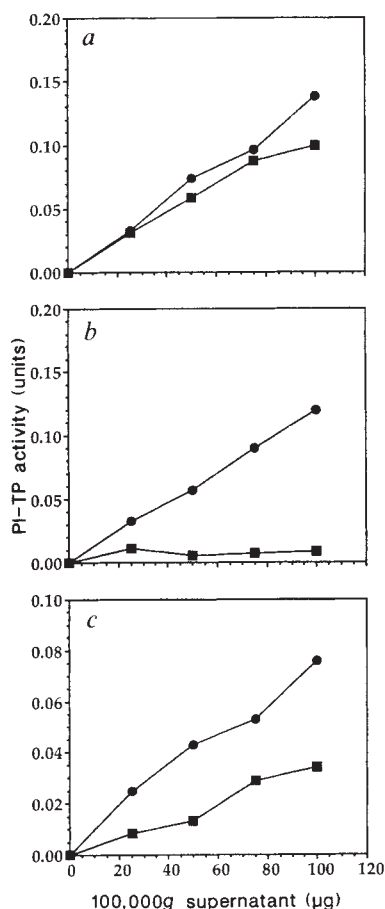


FIG. 1 PtdIns transfer protein (PI-TP) activity in the cytosol of wild-type (SEC14, ●) and *sec14-1^{ts}* (■) yeast strains.

METHODS. The isogenic yeast strains CTY182 (MATa, *ura3-52*, Δ *his3-200*, *lys2-801*, SEC14) and CTY1-1A (MATa, *ura3-52*, Δ *his3-200*, *lys2-801*, *sec14-1^{ts}*) were grown at 30 °C in 200 ml minimal medium (6.7 g l⁻¹ yeast nitrogen base, 20 g l⁻¹ glucose, 20 mg l⁻¹ histidine and uracil, 30 mg l⁻¹ lysine) to an absorbance at 600 nm of 1–1.5. The 100,000g supernatant fraction was prepared from each strain and assayed for PtdIns transfer as previously described³, with the following modifications. Assay mixtures containing phospholipid vesicles, buffer and the 100,000g supernatant fraction were either assayed directly at 25 °C (a) by addition of [³H]PtdIns-labelled microsomes, or preincubated at 37 °C for 15 min (b and c) before addition of [³H]PtdIns-labelled microsomes. Assays were carried out at 25 °C (a and c) or at 37 °C (b) for 30 min.

assayed at 25 °C but not at 37 °C. Thus, PtdIns transfer measured *in vitro* correlated directly with *sec14-1^{ts}* gene product function *in vivo*. By contrast, no PtdCh transfer activity could be detected in *sec14-1^{ts}* lysates at either 25 °C or 37 °C, even though the extracts from SEC14 and *sec14-1^{ts}*/pCEN-PIT1 strains had clearly measurable PtdCh transfer activity at both temperatures. The lack of each type of transfer activity at the nonpermissive temperature in the *sec14-1^{ts}* strain indicates that PtdIns transfer protein accounts for most of the PtdIns/PtdCh transfer activity in yeast.

What role could a phospholipid transfer protein have in the transport of intracellular protein? Existing evidence points to an execution point for the SEC14p late in the protein maturation pathway in the Golgi complex^{1,7,8}. It has been suggested that transfer proteins may be involved in lipid retrieval from the Golgi complex back to the endoplasmic reticulum thereby maintaining an optimal phospholipid/protein ratio for Golgi function⁹. Enzymes such as protein kinase C (ref. 10) and phospholipase A₂ (ref. 11) are dependent on the surface concentration and physical state of membrane components, so dilution by phospholipid might compromise the activity of membrane components of the compartment-specific secretion machinery. The exaggeration of the yeast Golgi complex in *sec14-1^{ts}* mutants¹, and the rapid onset of the corresponding secretory block² are consistent with this view.

An alternative hypothesis is that the SEC14p delivers PtdIns, a major acidic phospholipid in yeast synthesized in the endoplasmic reticulum¹², to late Golgi membranes for a specific function. Protein export from *Escherichia coli*^{13,14} and protein import into mitochondria¹⁵ depend on acidic phospholipids. The movement of PtdIns to the Golgi at the expense of PtdCh catalysed by the SEC14p could provide the acidic phospholipid content necessary to the catalysis of vesicle-mediated exit of proteins from the Golgi. Alternatively, the Golgi PtdIns may be the mediator of a lipid-mediated signalling pathway which serves

to regulate the metabolism of transport vesicles at the level of the late Golgi membranes, in direct analogy to the models formulated for the generation of second messengers from the turnover of PtdIns¹⁶.

Finally, the possibility still remains that phospholipid binding and not phospholipid transfer may be more directly related to the function of the SEC14p. This protein may be related to the class of cytosolic factors that mediate the metabolism of bulk carrier vesicles which move between the endoplasmic reticulum and the Golgi¹⁷. In this case, binding of either PtdIns or PtdCh would regulate the SEC14p-mediated exit of proteins from the Golgi.

We have demonstrated that the PtdIns/PtdCh transfer protein of yeast is essential for the compartment-specific stimulation of a late Golgi secretory process. These results should provide a foundation for a more detailed study of the largely uncharacterized involvement of phospholipids in secretory pathway function. In this regard we have obtained *S. cerevisiae* mutants that bypass the normally essential SEC14p requirement⁶. □

Received 21 June; accepted 6 August 1990.

- Novick, P., Field, C. & Schekman, R. *Cell* **21**, 205–215 (1980).
- Barkaitis, V. A., Malehorn, D. E., Ennr, S. D. & Greene, R. *J. Cell Biol.* **108**, 1271–1281 (1989).
- Aitken, J. F., van Heusden, G. P. H., Temkin, M. & Dowhan, W. *J. Biol. Chem.* **265**, 4711–4717 (1990).
- Wirtz, K. W. A. *Biochim. biophys. Acta* **344**, 95–117 (1974).
- Helmkamp, G. M. *J. Bioenergetics Biomembranes* **18**, 71–90 (1986).
- Cleves, A. E., Novick, P. J. & Barkaitis, V. A. *J. Cell Biol.* **109**, 2939–2959 (1989).
- Franzoso, A. & Schekman, R. *EMBO J.* **8**, 2695–2702 (1989).
- Stevens, T., Esmon, B. & Schekman, R. *Cell* **30**, 439–448 (1982).
- Weiland, F. T., Gleason, M. L., Serafini, T. A. & Rothman, J. E. *Cell* **50**, 289–300 (1987).
- Hannun, Y. A., Loomis, C. R. & Bell, R. M. *J. Biol. Chem.* **261**, 7184–7190 (1986).
- Plückthun, A. & Dennis, E. A. *J. Biol. Chem.* **263**, 11099–11106 (1988).
- Carman, G. M. & Henry, S. A. *A. Rev. Biochem.* **58**, 635–669 (1989).
- Lill, R., Dowhan, W. & Wickner, W. *Cell* **60**, 217–280 (1990).
- de Vrije, T., de Swart, R. L., Dowhan, W., Tommassen, J. & de Kruijff, B. *Nature* **334**, 173–175 (1988).
- Endo, T., Eilers, M. & Schatz, G. *J. Biol. Chem.* **264**, 2951–2956 (1989).
- Berridge, M. J. *A. Rev. Biochem.* **58**, 159–193 (1987).
- Warren, G. *Nature* **345**, 382–383 (1990).

Novel potential mitotic motor protein encoded by the fission yeast *cut7⁺* gene

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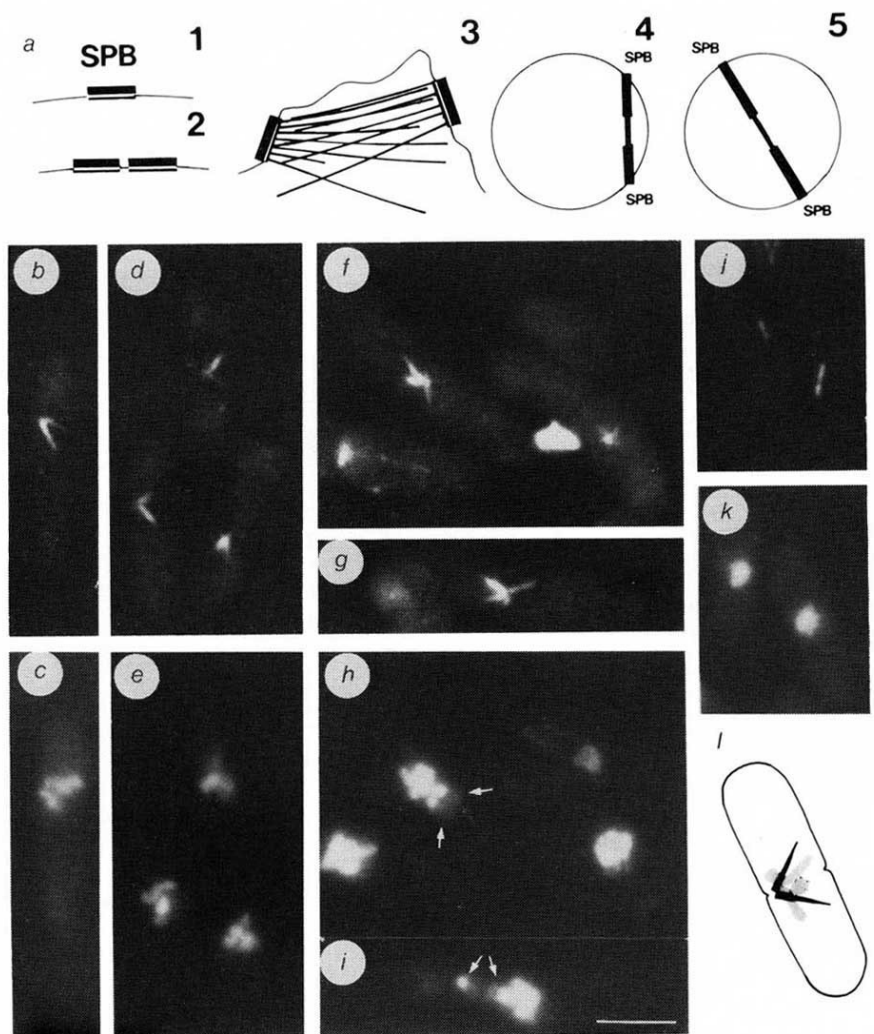
THE structure equivalent to higher eukaryotic centrosomes in fission yeast, the nuclear membrane-bound spindle pole body, is inactive during interphase. On transition from G2 to M phase of the cell cycle, the spindle pole body duplicates; the daughter pole bodies seed microtubules which interdigitate to form a short spindle that elongates to span the nucleus at metaphase¹⁻⁵. We have identified two loci which, when mutated, block spindle formation. The predicted product of one of these genes, *cut7⁺* (ref. 6), contains an amino-terminal domain similar to the kinesin heavy chain head domain^{7,8}, indicating that the *cut7⁺* product could be a spindle motor. The *cut7⁺* gene resembles the *Aspergillus nidulans* putative spindle motor gene *bimC* (ref. 9), both in terms of its organization with a homologous amino-terminal head and no obvious heptad repeats and in the morphology of the mutant phenotype. But we find no similarity between the carboxy termini of these genes, suggesting that either the *cut7⁺* gene represents a new class of kinesin genes and that fission yeast may in addition contain a *bimC* homologue, or that the carboxy termini of these mitotic

kinesins are not evolutionarily conserved and that the *cut7⁺* gene belongs to a subgroup of *bimC*-related kinesins.

We identified two spindle-formation mutants exhibiting essentially identical phenotypes at the restrictive temperature of 36 °C by screening *Schizosaccharomyces pombe* temperature-sensitive (ts) mutant bank¹⁰ by anti-tubulin immunofluorescence microscopy. One maps to the *cut7* locus on chromosome I⁶, whereas the other, *ts549*, maps to a different locus on chromosome II (within 1.3 cM of *cut2* (ref. 6 and S. Uzawa, unpublished data)). Instead of forming a normal mitotic spindle (Fig. 1j, k), these mutants (*cut7-332* and *ts549*), and the originally identified *cut7* allele (*cut7-446*), exhibit a novel V-shaped staining pattern at 36 °C (Fig. 1b, d). Microtubules end abruptly at the base of each arm of the V whereas at the distal end they seem to taper away. The V is always associated with the chromosomal domain (Fig. 1l). This phenotype is very different from that of *cut1* mutants which affect the spindle pole body structure and number¹¹. The ts lethality of the *cut7-446* mutation is semidominant (heterozygous diploids can grow at 30 °C, a restrictive temperature for haploid *cut7-446* cells, but cannot grow at 36 °C) whereas that in the *ts549* strain is recessive in a heterozygous diploid state (data not shown).

We examined the nature of the *cut7* defect in detail. A temperature shift of a culture of *cut7-446* cells synchronized in cell cycle progression indicates the timing of events during the abortive mitosis (Fig. 2). Cell viability was unaffected during interphase (up to 105 min), but at the time when wild-type cells would normally enter mitosis and before cell division is initiated, it radically decreases. Coincidentally, cytoplasmic microtubules,

FIG. 1 Immunofluorescence microscopy of *cut7* and *ts549* mutants. **a**, A diagram of the events leading to spindle formation in wild-type cells. The initially single interphase spindle pole body (SPB) (1) duplicates on the transition of the G2/M boundary (2) and the two daughter SPBs are activated and seed microtubules (3). Microtubule interdigitation and SPB separation occur coincidentally and a short prometaphase spindle (5) forms by extension of the prophase spindle (4). Both ends of the metaphase spindle are slightly broader than the middle, presumably because of pole-to-kinetochore microtubules^{4,5}. **b-k**, Comparison of mutant and wild-type images of mitotic microtubules. **b, d, f, h, j**, Anti-tubulin immunofluorescence; **c, e, g, i, k**, Diamidinophenyl indole DNA staining. **b, c**, A cell from an asynchronous *ts549* culture after 4.5 h at 36 °C. **d, e**, cells of a synchronous *cut7-446* culture 100 min after combined synchronization and shift to 36 °C. **f, g** and **h, i**, Cells of the same *cut7-446* culture 300 min after the temperature shift. Multiple anti-tubulin staining bars are visible along with two chromosome IIIs (arrows), identified by the two ribosomal gene cluster bars extending into the nucleolus from each small diamidinophenyl indole-staining body¹⁶, suggesting that a second cell cycle is in progress. **j, k**, Typical wild-type prophase (upper) and metaphase (lower) spindle; **l**, shows a cartoon of the *cut7⁻* and *ts549⁻* phenotype. Bar 5 µm. **METHODS**. Cells were grown in rich YPD medium. Cell cycle synchronization was carried out according to the sucrose density gradient technique of Mitchison¹⁷. YOL1/34 (ref. 18) was used for immunofluorescence⁴.



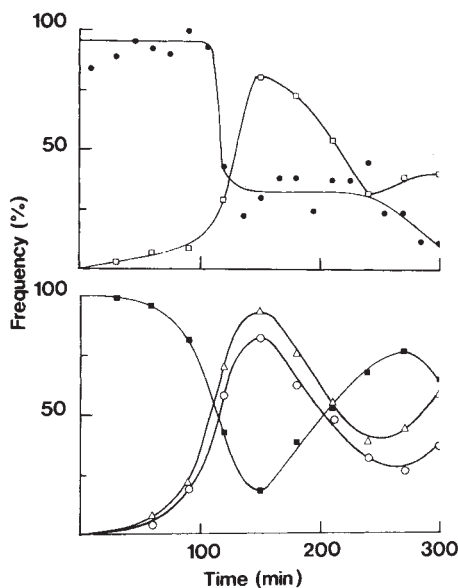


FIG. 3 The nucleotide and predicted amino-acid sequence of the *cut7⁺* gene. *a*, Rescue of both *cut7* alleles by subclones of the 7-kilobase (kb) *SacI cut7⁺* containing fragment. +, Complementation; nt, no transformants obtained even when transformed into wild type cells; -, no complementation. *b*, The complete nucleotide sequence of the insert SKa78. A 3,260 open reading frame (ORF) encoding a protein of *M_r* 120,726 starting at -6 with the first ATG at position 1 is interrupted by an intron at nucleotide 368 (consensus sequences are underlined twice). Homology comparisons between *cut7⁺* and *bimC* are consistent with the allocation of the intron and ATG. The key residues in a C-terminal direct repeat; DXSLXLETTXE/D single-letter amino-acid notation between positions 975-998 are underlined.

METHODS. *cut7-446* cells were transformed with a genomic DNA bank²⁰ and assayed for complementation of the temperature-sensitive phenotype. Two unstable transformants out of an estimated 10,000 grew at both 25 and 36 °C. Both contained the same 14-kb insert, which was subcloned to a minimally complementing 7-kb *SacI* fragment (*cut7⁺.4*) which was shown to be continuous in the genome by genomic Southern hybridization. It was shown to contain the *cut7⁺* gene by: *NotI/LEU2* integration mapping to the correct region of the *cut7⁺* containing 1,000-kb chromosome I *NotI* fragment²¹; subsequent tetrad analysis of this strain (PD:NPD:TT = 24:0:0); gene conversion, and the fact that the 7-kb fragment also contains the *his1⁺* gene (to be presented elsewhere) which, by classical genetic analysis, maps near to *cut7⁺* (ref. 6). *ExoIII* nested sequencing deletions were constructed in Bluescript (Stratagene). Inserts from some of these were subcloned and tested for complementation.

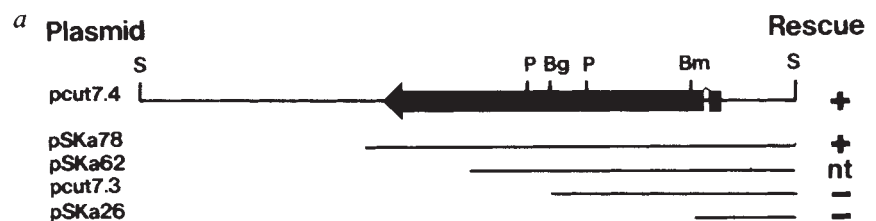
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FIG. 2 Quantitative synchronous culture analysis of a *cut7-446* culture at the restrictive temperature. G2 cells were inoculated at time 0 into fresh YPD medium at 36 °C. (Examples of cells from this culture are shown in Fig. 1*d-i*). ●, Cell viability; □, presence of septa (the division machinery analogous to the cleavage furrows of higher eukaryotes); △, condensed chromosomes; ■, interphase microtubules; ○, mitotic microtubules. METHODS. Cells were grown in YPD medium at 25 °C, synchronized¹⁷ and inoculated into fresh YPD medium at 36 °C at time 0. Cell viability was determined by plating at 25 °C, cell plate index by calcofluor staining¹⁹, chromosome condensation index by diamidinophenyl indole staining¹⁶ and microtubule configurations by YOL 1/34 anti-tubulin immunofluorescence⁴.

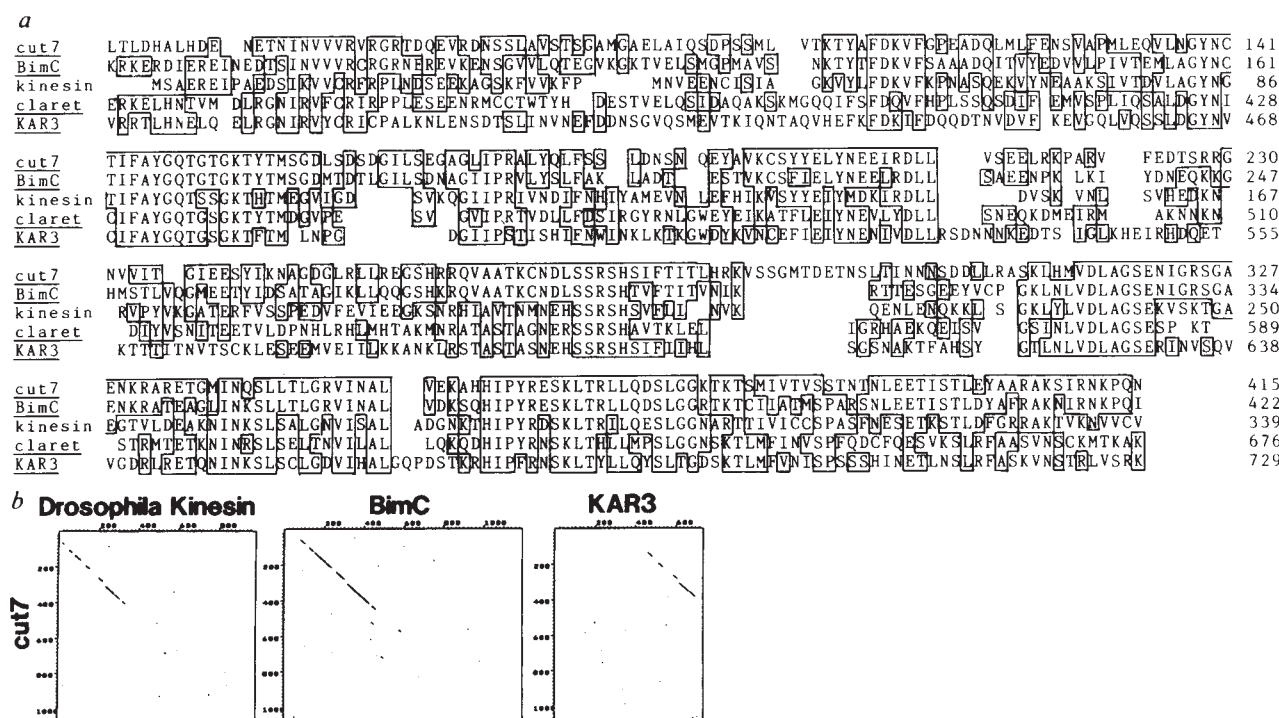


FIG. 4 A homology comparison of the *cut7*⁺ predicted gene product with those of the *Aspergillus nidulans* *bimC*, budding yeast *KAR3* and *Drosophila melanogaster* kinesin heavy chain and *claret*nd genes. *a*, An alignment of the *cut7*⁺, *bimC*, *KAR3*, *Drosophila* kinesin and *claret*nd predicted amino-acid sequences. The names and position relative to the first residue are indicated

markers of the interphase state, disappear and are replaced by the typical *cut7* V staining pattern and not by the normal prometaphase spindle (Fig. 1j, k). On further incubation, chromosomes decondense and cells containing interphase microtubules reappear, peaking around 270 min, indicating that cell-cycle progression is not blocked. This conclusion is confirmed by the appearance after 300 min at 36 °C of large cells with two sets of chromosomes and multiple anti-tubulin staining bars (Fig. 1f-i).

Given the *cut7*⁺ sequence homology data presented below, a plausible interpretation of the mutant phenotype is that spindle pole body duplication is occurring with each entry into mitosis but that the seeded microtubules are unable to interdigitate and subsequently slide apart to form the spindle (Fig. 1a, step 3). Instead, the microtubules extend away from the spindle pole bodies at the base of the V and nuclear separation is arrested.

We isolated a single genomic DNA that could rescue the *ts* lethality of both the *cut7*-446 and *cut7*-332 alleles (but not *ts549*) and which encodes the *cut7*⁺ gene (as shown by homologous integration). Nucleotide sequence determination of the minimally complementing subclone, pSKa78 (Fig. 3a) reveals a large coding region, interrupted by one intron, encoding a 1,073-residue protein (predicted relative molecular mass 120,726 (*M_r*, 21K); Fig. 3b) which shares structural similarity with *Drosophila* and squid kinesin heavy chains (Fig. 4b)^{7,8}. Consistent with the semidominant nature of the *cut7*-446 defect, a truncated version of the gene on a high copy number plasmid is toxic to wild-type cells (Fig. 3a). We are now constructing deletion strains to investigate the phenotype of the null mutant.

The N-terminal 415 amino acids of the predicted *cut7*⁺ product, including a potential ATPase site, share 39% identity with the 339 N-terminal 'globular head' domain residues of *Drosophila melanogaster* kinesin heavy chain⁷ (Fig. 4a, b), suggesting that the *cut7*⁺ gene product could be a microtubule-based ATP-dependent translocator. Following this there is a stretch extending from position 415 to 945 containing only one

on the left and right side of each sequence respectively. Over the regions shown, *cut7*⁺ and *bimC* are most homologous with 57% identity. *b*, Harr plots comparing the *cut7*⁺ amino-acid sequence with those of *Drosophila* kinesin heavy chain, *BimC* and *KAR3* as indicated. The window for comparison is >8 identical residues in 20 continuous amino acids.

proline at position 663. This region does not contain an obvious heptad repeat motif.

The *cut7*⁺ globular head domain has a similar sequence to all the identified kinesin heavy chain-related genes (the highest identity, 57%, being with the *bimC* gene product) (Fig. 4a). Although the gene organization of *cut7*⁺ resembles that of squid and *Drosophila* kinesin heavy chains^{7,8} and the *Aspergillus nidulans* mitotic gene *bimC* (ref. 9), it is different from the smaller homologues, *KAR3* (ref. 12) and *claret*nd (refs. 13, 14), in which the motor domain is C-terminal (Fig. 4b).

The similarity between *cut7*⁺ and *bimC* extends beyond sequence comparisons, gene organization and the fact that of the kinesin homologues only these two do not contain a central heptad repeat motif (as a mutation in the *bimC* gene blocks spindle function in a similar way to *cut7* and *ts549* mutations with duplicated spindle pole bodies lying at the base of extending microtubule arrays). Although these similarities could suggest a similar function for the *cut7*⁺ and *bimC* gene products, the extreme C termini of these genes (a region of kinesin known to interact with other proteins^{7,15}) share no significant homology (Fig. 4b). This is in contrast to a comparison between *Drosophila* and squid kinesins, where extensive similarity between the C termini has been reported⁸, raising the possibility that *cut7*⁺ and *bimC* may not be functionally interchangeable. As such, the *cut7*⁺ gene product may represent a new class of kinesin-related proteins to add to the four described to date: kinesin, *KAR3*, *bimC* and *claret*nd. If true, this conclusion suggests that there may be a genuine *bimC* gene in *S. pombe* in a manner analogous to the tubulin gene family, and that the phenotype described here can be indicative of a 'motorless' mutant. Alternatively, the *cut7*⁺ and *bimC* genes may represent isotypes of the same mitotic kinesin-related molecule, which has not been subject to strong evolutionary conservation. □

Received 5 June; accepted 20 July 1990.

1. McCully, E. K. & Robinow, C. F. *J. Cell Sci.* **9**, 475-507 (1971).
2. Tanaka, K. & Kanbe, T. *J. Cell Sci.* **80**, 253-268 (1986).

3. Kanbe, T., Hiraoka, Y., Tanaka, K. & Yanagida, M. *J. Cell Sci.* **96**, 275–283 (1990).
4. Hagan, I. M. & Hyams, J. S. *J. Cell Sci.* **89**, 343–357 (1989).
5. Hiraoka, Y., Toda, T. & Yanagida, M. *Cell* **39**, 349–358 (1984).
6. Hirano, T., Funahashi, S., Uemura, T. & Yanagida, M. *EMBO J.* **5**, 2973–2979 (1986).
7. Yang, J. T., Layman, R. A. & Goldstein, L. S. B. *Cell* **56**, 879–889 (1989).
8. Kosik, K. S., Orecchio, L. D., Schnapp, B., Inouye, H. & Neve, R. L. *J. Biol. Chem.* **265**, 3278–3283 (1990).
9. Enos, A. P. & Morris, N. R. *Cell* **60**, 1019–1027 (1990).
10. Uemura, T. & Yanagida, M. *EMBO J.* **3**, 1737–1744 (1984).
11. Uzawa, S., Samejima, I., Hirano, T., Tanaka, K. & Yanagida, M. *Cell* (in the press).
12. Meluh, P. B. & Rose, M. D. *Cell* **60**, 1029–1041 (1990).
13. Endow, S. A., Henikoff, S. & Soler-Niedziela, L. *Nature* **345**, 81–83 (1990).
14. McDonald, H. B. & Goldstein, L. S. B. *Cell* **61**, 991–1000 (1990).
15. Hirokawa, N. et al. *Cell* **56**, 867–878 (1989).
16. Toda, T., Yamamoto, M. & Yanagida, M. *J. Cell Sci.* **52**, 271–287 (1981).
17. Mitchison, J. M. *Meth. Cell Physiol.* **4**, 131–154 (1970).
18. Kilmartin, J. V., Wright, J. B. & Milstein, C. *J. Cell Biol.* **93**, 576–582 (1982).
19. Streiblova, E. & Beran, K. *Exp. Cell Res.* **30**, 603–610 (1963).
20. Beach, D., Durkacz, B. & Nurse, P. *Nature* **300**, 706–709 (1982).
21. Fan, J.-B. et al. *Nucleic Acids Res.* **17**, 2801–2818 (1988).

ACKNOWLEDGEMENTS: We are grateful to J. V. Kilmartin for his gift of YOL1/34 antibody and thank M. Rose, P. B. Meluh, A. P. Enos and N. R. Morris for communicating information before publication. We also thank S. Uzawa for discussions. This work was supported by the Ministry of Education, Science and Culture of Japan (M.Y.) and a Japan Society for the Promotion of Science Fellowship to I.H.

Formation of amyloid-like fibrils in COS cells overexpressing part of the Alzheimer amyloid protein precursor

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A PATHOLOGICAL hallmark of Alzheimer's disease is the deposition of amyloid fibrils in the brain. The principal component of the amyloid fibril is β /A4 protein^{1,2}, which is derived from a large membrane-bound glycoprotein, Alzheimer amyloid protein precursor (APP)³. Although the deposition of amyloid is thought to result from the aberrant processing of APP, the detailed molecular mechanisms of amyloidogenesis remain unclear. A C-terminal fragment of APP which spans the β /A4 and cytoplasmic domains has a tendency to self-aggregate^{4,5}. In an attempt to establish a cultured-cell model for amyloid fibril formation, we have transfected COS-1 cells with complementary DNA encoding the C-terminal 100 residues of APP. In the perinuclear regions of a small population of DNA-transfected cells, we observed inclusion-like deposits which showed a strong immunohistochemical reaction towards an anti-C-terminal APP antibody or an anti- β /A4 amyloid core-specific antibody. Electron microscope observations of the inclusion-carrying cells revealed an accumulation of amyloid-like fibrils of 8–22 nm diameter near and on the nuclear membrane. The fibrils showed a beaded or helical structure, and reacted positively with the anti-C-terminus antibody by immunoelectron microscopy. These results suggest that the formation of amyloid fibrils is an inherent characteristic of the C-terminal peptide of APP. The present system provides a suitable model for the molecular dissection of the process of brain amyloidogenesis.

We made two cDNA constructs for the expression of APP-C100 (Fig. 1a); h β was designed for expressing only the exact region of APP-C100, and sh β for expressing the APP-C100 region plus a signal peptide for secretion and membrane integration. The ATG codon flanking the 5'-end of the β /A4 region in h β (corresponding to position 596 of APP695) was used as an initiation codon to synthesize the subsequent 99 amino acids of the C-terminal peptide of APP (APP-C100), whereas the transla-

tion initiation codon in sh β corresponds to that of rat preproenkephalin A cDNA⁶. These hybrid cDNAs were inserted into the vector p91023(B), a highly efficient expression vector having an SV40 origin and an adenovirus promoter⁷, and transfected into COS-1 cells (African green monkey kidney cells). Transient expression of APP-C100 in the COS-1 cells was examined by immunoblotting and immunoprecipitation methods using an anti-C-terminal antibody raised against residues 681–695 of APP⁸ (Fig. 1b and c). On SDS-polyacrylamide gels, cell extracts of both h β - and sh β -transfected COS-1 cells contained a major band corresponding to APP-C100 with an apparent relative molecular mass of 16,000 (M_r 16K); the level in sh β -transfected cells was about fivefold higher than that of h β -transfected cells (Fig. 1b). A band of APP-C100 with M_r 16K was also detected in the conditioned medium of sh β -transfected cells but not of h β -transfected cells (Fig. 1c), indicating the efficacy of the signal peptide of the sh β -directed protein and the intracellular containment of APP-C100 in h β -transfected cells.

We then carried out immunocytochemical staining of the DNA-transfected COS-1 cells with the anti-C-terminal antibody (Fig. 2). About 20% of the h β -transfected cells were clearly immunoreactive. Unexpectedly, 10–20 μ m diameter inclusion-like deposits were found in perinuclear regions of a subpopulation of these immunoreactive cells (4% of total cells) (Fig. 2a). The inclusion-carrying cells often showed deformation of the nucleus, suggesting a possible noxious effect of the inclusion on this organelle. Similarly, about 20% of sh β -transfected COS-1 cells were positively stained with the anti-C-terminus antibody. The reticular distribution of the immunoreactivity in sh β -transfected cells suggested the successful integration of expressed APP-C100 into membranes (Fig. 2b). Only ~1% of the sh β -transfected cells contained the inclusion, however; this may be because the secretion and membrane integration of APP-C100 in the sh β -transfected cells resulted in reduced cytoplasmic accumulation. The inclusion-like structures in h β -transfected cells also reacted with an antibody against synthetic β /A4 peptide (residues 1–24) (Fig. 2c), which has been shown to react specifically with amyloid cores of senile plaques⁸.

We then characterized the intracellular inclusions in h β -transfected COS-1 cells by electron microscopy. In a single selected cell carrying an inclusion-like deposit, there was an accumulation of filamentous structures having varying diameters (8–22 nm); a beaded or helical structure of these filaments is apparent (Fig. 3a and b). The deposition of fibrils near and on the nuclear membrane was observed, and rods with a hollow structure were occasionally visible (Fig. 3c). These structures resemble amyloid fibrils in the brain amyloid^{9–13} and amyloid-like fibrils generated *in vitro* with a synthetic peptide consisting of residues 1–28 of β /A4 protein¹⁴, although in these cases the fibrils are 4–11 nm in diameter. However, the amyloid-like fibrils in h β -transfected cells are likely to contain the C-terminal region of APP in addition to β /A4 peptide as the anti-C-terminus antibody reacted with the amyloid-like structures near and on the nuclear membrane (Fig. 3d). Thus, it is likely that the C-terminal sequence of APP-C100 remains undeleted in most, if not all, the amyloid-like fibrils.

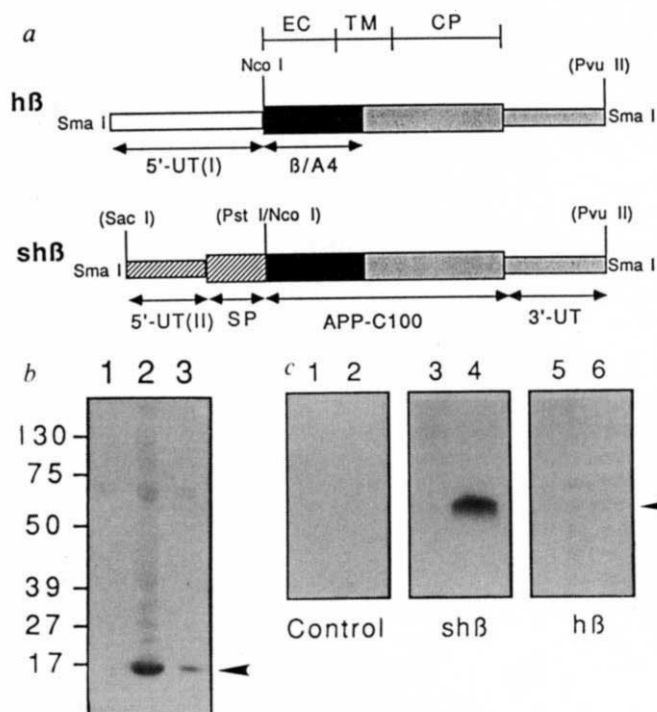
APP may be cleaved in the interior of the β /A4 domain under physiological conditions^{15,16} to produce secretory forms of APP, and an abnormal or alternative cleavage pattern must give rise to the amyloid formation in Alzheimer's disease. The present finding indicates that a single abnormal cleavage of intact APP near the N terminus of the β /A4 protein domain, which would generate an N-terminal truncated peptide similar to APP-C100, could potentially form amyloid-like fibrils as long as the amyloidogenic β /A4 sequence remains intact. This raises the possibility that the proteolytic cleavage to release the β /A4 peptide (M_r ~4K) from APP may occur at a later stage in amyloidogenesis after the formation of fibrous structures.

The deposition of senile plaque amyloid occurs in the extracellular space of the brain in Alzheimer's disease or related dis-

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FIG. 1 *a*, Constructs for expression of the C-terminal 100 residues of APP (APP-C100). The coding region (enlarged bar) of APP-C100 consists of extracellular (EC), transmembrane (TM), and cytoplasmic (CP) domains of APP. The derivations of sequences are: 5'-UT(I), 5'-untranslated region of rabbit slow muscle Ca²⁺-ATPase cDNA; 5'-UT(II), 5'-untranslated region of rat preproenkephalin cDNA; 3'-UT, 3'-untranslated region of human APP; SP, signal peptide encoded in rat preproenkephalin cDNA. The β /A4 domain is shown as a solid bar. Also indicated are the restriction sites used in constructing the hybrid sequences. The restriction sites in parentheses are the original sites converted to other restriction sites for ligation. *b*, Immunoblotting of APP-C100 expressed in h β - and sh β -transfected COS-1 cells. APP-C100 expressed in DNA-transfected COS-1 cells was analysed by immunoblotting. Lane 1, mock transfected cells; lane 2, cells transfected with sh β ; lanes 3, cells transfected with h β . *M*, size markers (in *M*) at left. Arrowhead, 16K. *c*, Immunoprecipitation of APP-C100 secreted into the conditioned medium of h β - and sh β -transfected COS-1 cultures. Proteins synthesized in the DNA-transfected cells were labelled with [³⁵S]methionine. APP-C100 secreted into the culture medium was immunoprecipitated with anti-C-terminal antibody. Panels: Control, mock-transfected cells; sh β , cells transfected with sh β ; h β , cells transfected with h β . Lanes 1, 3, and 5, samples precipitated with preimmune serum; lanes 2, 4, and 6, samples precipitated with anti-C-terminal peptide of APP. Arrowhead, 16K. The value of 16K for immunoreactive APP-C100 (*b* and *c*) is larger than the calculated size (11.3K).

METHODS. *a*, APP-C100 cDNA and its flanking 3'-untranslated region was excised as an *EcoRI*-*PvuII* fragment of clone B2.3 (ref. 22) and cloned into *EcoRI*/*SmaI* sites of Bluescript SK (+) (Stratagene); the *EcoRI*-*PvuII* fragment corresponds to positions 1,796–2,219 of APP695 cDNA³. *NcoI* site containing the initiation ATG codon was constructed at the 5'-end of the *EcoRI*-*PvuII* fragment by replacing 5'-GATAAGCTTGATATCGAATTC-3' with 5'-GATACCATGGATGCGAATTC-3' by oligonucleotide-directed site-specific mutagenesis²³. The *Bam*HI site, which adjoins the ligated *PvuII*/*SmaI* site in the multiple cloning sites of Bluescript, was converted to a *SmaI* site by adding *SmaI* linker to the blunted end. 0.4-kilobase (kb) of *NcoI*-*SmaI* fragment, which contains the coding region of APP-C100 and 3'-untranslated region, was then excised. *SmaI*-*NcoI* fragment (positions –215–2) of rabbit slow muscle Ca²⁺-ATPase cDNA²⁴ (gift from D. H. MacLennan, University of Toronto) (5'-UT(I)) was ligated to *NcoI*-*SmaI* fragment (h β). To add a signal sequence (SP) to APP-C100, a 183-bp *SacI*-*PstI* fragment of rat preproenkephalin cDNA⁶ was ligated to 5'-end of *NcoI*-*SmaI* fragment after blunting both *PstI* and *NcoI* sites (sh β). An in-frame arrangement of the ligated site was confirmed by the dideoxy-chain termination method²⁵. The *SacI* site at the 5'-end of 5'-UT(II) was blunted and converted to a *SmaI* site by linker attachment. The unique cloning site of *EcoRI* in the sequence of expression vector p91023(B) (ref. 7, gift from R. J. Kaufman, Genetics Institute, Boston) was converted to a *SmaI* site by adding *SmaI* linker to the blunted end. These constructs of h β and sh β , having *SmaI* sites at both ends, were inserted into the p91023(B) vector at a newly constructed *SmaI* site of the vector. *b*, COS-1 cells were maintained in DMEM supplemented with

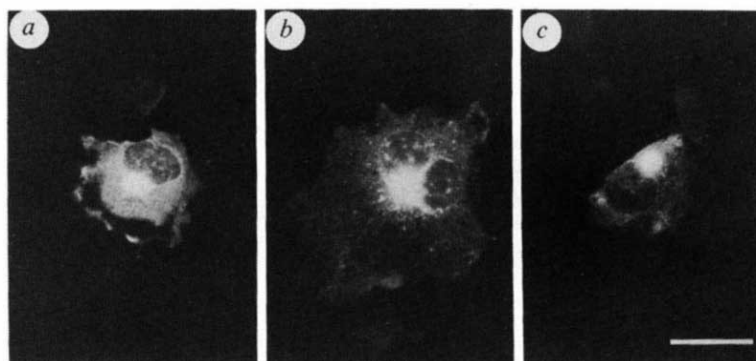


nonessential amino acids, L-glutamine, antibiotics, and 10% FCS under 5% CO₂/95% air at 37 °C. DNA was transfected into COS-1 cells according to the DEAE-dextran method²⁶ followed by chloroquine-shock treatment under the conditions described previously²⁷. The cells were incubated for 72 h, collected and lysed in buffer containing 0.65 M Tris-HCl (pH 6.5), 2% SDS, 10% glycerol and 5% 2-mercaptoethanol. Samples were then electrophoresed on SDS-polyacrylamide gels (15% acrylamide)²⁸ and immunoblotted with an antibody against the C-terminal (residues 681–695) of APP695 (1:100)⁸, followed by detection using the avidin-biotin-peroxidase complex technique with a kit and the procedure recommended by Vector Labs; peroxidase activity was visualized by reaction with 4-chloro-1-naphthol. *c*, DNA-transfected cells were incubated for 48 h, and the medium was replaced with serum- and methionine-free medium. Proteins synthesized were labelled for 3 h by adding [³⁵S]methionine (Amersham) at 250 μ Ci ml⁻¹ of culture medium. [³⁵S]-labelled APP-C100 excreted into the medium was immunoprecipitated with anti-C-terminal antibody (1:100) and protein A-bacterial adsorbent (Seikagaku-Kogyo) as described²⁹. The precipitate was analysed on SDS-PAGE (15% acrylamide) followed by detection on autoradiography (16-h exposure).

orders. The present cell model of intracellular fibril formation is, however, of great utility for molecular studies of amyloidogenesis. One of the morphological forms of amyloid-like fibrils in the transfected COS-1 cells may be similar to amyloid fibrils *in vivo*. The deposition of amyloid-like structures near and on the nuclear membrane suggests the involvement of membranous structures in the initiation of fibril formation

and/or development of the fibrils. Some intracellular proteins may interact with developing fibrils, resulting in modulation or decoration of the filaments. This may account for the variable sizes in the diameter of amyloid-like structures in the h β -transfected COS-1 cells. In addition, the formation of amyloid-like fibrils in the DNA-transfected cells can be manipulated by various experimental procedures; amyloid-like structures may

FIG. 2 Fluorescent micrographs of h β - and sh β -transfected COS-1 cells. DNA-transfected COS-1 cells were cultured for 72 h on gelatin-coated glass coverslips, and then fixed with 90% methanol for 10 min and with methanol-acetone (1:1) for 15 min at –20 °C. Fixed cells were incubated with the anti-C-terminus antibody (1:1,000) or an antibody against synthetic β /A4 protein (1–24) (1:1,000)⁸ in TBS (10 mM Tris-HCl, 0.9% NaCl, pH 7.5) for 60 min at 37 °C. After washing with TBS, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit immunoglobulins (G+L) (1:80) (Tago) for 30 min at 37 °C. After washing with TBS, immunoreactive cells were visualized and photographed with a fluorescent microscope (Nikon Microphot FXA-FL) using Kodak TMY-400 film. *a*, h β -transfected cell, stained with anti-C-terminus antibody; *b*, sh β -transfected cell, stained with anti-C-terminus antibody; *c*, h β -transfected cell, stained with anti- β /A4(1–24) antibody. Note the intensely immunoreactive inclusion-like



deposits in the perinuclear region. Scale bar, 50 μ m for *a*–*c*.

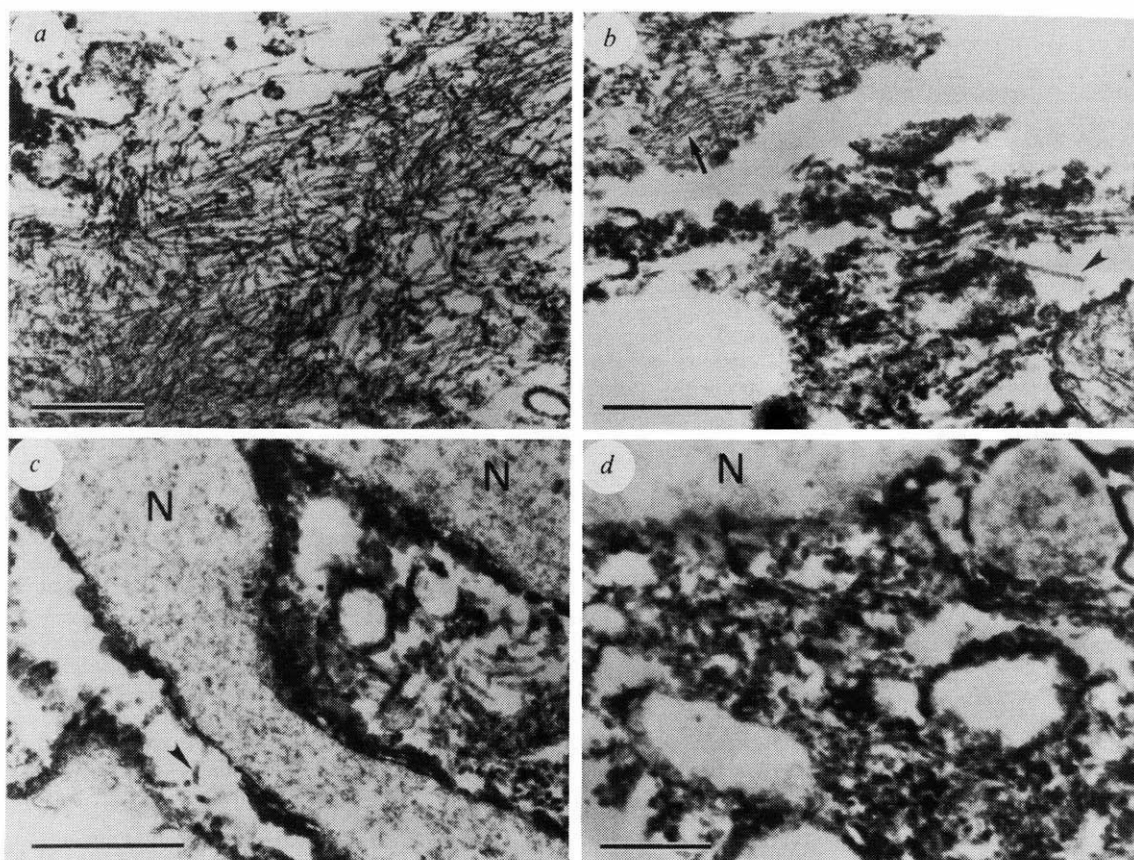


FIG. 3 Electron micrographs of h β -transfected COS-1 cells having inclusion-like deposits. COS-1 cells transfected with h β were stained with the anti-C-terminal antibody followed by detection with 3,3'-diaminobenzidine (DAB) peroxidase substrate. Ultrathin sections of selected cells showing intense immunoreactivity were observed under a transmission electron microscope. *a-c*, Sections stained with lead citrate and uranyl acetate. Note the accumulation of amyloid-like fibrils with a relatively homogeneous diameter of 15 nm (*a*) or with varying diameters from 8 nm (*b*, arrowhead) to 22 nm (*b*, arrowhead). These structures were found in the perinuclear region. Parallel rods having an electronlucent centre (*c*, arrowhead) and deposition of fibril-like structures were observed near and on the nuclear membrane (*c*, N). No such structures were found in mock-transfected COS-1 cells. *d*, Unstained section. Fibrils near the nuclear membrane are stained very darkly with the immunoperoxidase reaction products, indicating the presence of the C-terminal sequence in the amyloid-like fibrils, N, nucleus. Scale bars, 500 nm. METHODS. COS-1 cells were cultured on plastic coverslips and transfected

with h β . Cells were fixed in 1% formaldehyde in PBS for 30 min at room temperature. After washing with PBS, cells were treated with 2% goat serum for blocking, and incubated with the anti-C-terminus antibody (1:1,000) for 1 h at 37 °C. After washing the PBS, biotinylated anti-rabbit IgG and peroxidase-conjugated avidin were added. After fixing with 2.5% glutaraldehyde in PBS, cells were stained using the avidin-biotin-peroxidase technique with the kit and method recommended by Vector Labs. Peroxidase activity was visualized by reaction with 0.05% DAB and 0.01% H₂O₂ for 10 min. DAB-stained samples were postfixed in 2% OsO₄ in PBS for 1 h at room temperature. These samples were dehydrated and embedded in Araldite (Ciba-Geigy). Coverslips were removed after hardening of the resin, and single cells showing intense DAB staining were selected by trimming off the excess resin with a razor blade under a dissection microscope. Ultrathin sections, either unstained or stained with lead citrate and 2% (w/v) aqueous uranyl acetate, were observed under a Hitachi H700H electron microscope at 100 kV.

be modified by cotransfecting cloned cDNAs encoding various kinds of enzymes and other proteins (for example, proteases, protease inhibitors, protein kinases). It may also be feasible to modulate the process of fibril formation by drugs or chemicals. Such studies may provide valuable information on strategies for the prevention of amyloid formation designed to retard the progression of Alzheimer's disease. Furthermore, this COS-1 model presents a suitable system for defining the amino-acid sequences responsible for the formation of amyloid fibrils. Experiments using various DNAs mutated *in vitro* (carrying, for example, site-specific mutations in the β /A4 domain, deletion or substitution of membrane and cytoplasmic domains) can be carried out to examine their amyloidogenic potencies.

Yankner *et al.*¹ have shown that stable transfectants of pheochromocytoma (PC12) cells expressing APP-C100 gradually degenerate when induced to differentiate into neuronal cells by nerve growth factor. Here we have indicated that the dense deposition of amyloid-like fibrils near and on the nuclear membrane might lead to abnormal nuclear function. As it is difficult to estimate the cytotoxic effect of APP-C100 or of amyloid-like

fibril formation on the COS-1 cells in the present transient expression system, we are planning to use neural cell lines for examining some pathological effects of intracellular amyloid-like fibrils.

There are several preceding reports on the expression of APP in DNA transfected cells^{5,15,17-21}. None of them, however, have succeeded in demonstrating amyloid fibril-like structures. The main reason for our success is the selection of a transient expression system, rather than a stable system, using COS cells and the highly efficient expression vector p91023 in combination. The present transient expression system, which forms amyloid-like structures within 72 h, is an indispensable tool for studying the molecular basis of amyloidogenesis. The resulting information will lead to a better understanding of the pathogenesis of Alzheimer's disease and related disorders. □

Received 19 July; accepted 20 August 1990.

1. Glenner, G. G. & Wong, C. W. *Biochem. biophys. Res. Commun.* **122**, 1131-1135 (1984).
2. Masters, C. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 4245-4249 (1985).
3. Kang, J. *et al. Nature* **325**, 733-736 (1987).

4. Dyrks, T. *et al.* *EMBO J.* **7**, 949-957 (1988).
5. Wolf, D. *et al.* *EMBO J.* **9**, 2079-2084 (1990).
6. Yoshikawa, K., Williams, C. & Sabol, S. L. *J. Biol. Chem.* **259**, 14301-14308 (1984).
7. Wong, G. G. *et al.* *Science* **228**, 810-815 (1985).
8. Ishii, T., Kametani, F., Haga, S. & Sato, M. *Neuropath. appl. Neurobiol.* **15**, 135-147 (1989).
9. Kidd, M. *Brain* **87**, 307-320 (1964).
10. Terry, R. D., Gonatas, N. K. & Weiss, M. *Am. J. Pathol.* **44**, 269-283 (1964).
11. Merz, P. A. *et al.* *Acta neuropathol.* **60**, 113-124 (1983).
12. Miyakawa, T., Katsuragi, S., Watanabe, K., Shimoji, A. & Ikeuchi, Y. *Acta neuropathol.* **70**, 202-208 (1986).
13. Miyakawa, T., Watanabe, K. & Katsuragi, S. *Virochows Arch.* **52**, 99-106 (1986).
14. Kirschner, D. A. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **84**, 6953-6957 (1987).
15. Sisodia, S. S., Koo, E. H., Beyreuther, K., Unterbeck, A. & Price, D. L. *Science* **248**, 492-495 (1990).
16. Esch, F. S. *et al.* *Science* **248**, 1122-1124 (1990).
17. Yankner, B. A. *et al.* *Science* **245**, 417-429 (1989).
18. Selkoe, D. J. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **85**, 7341-7345 (1988).
19. Weidemann, A. *et al.* *Cell* **57**, 115-126 (1989).
20. Saitoh, T. *et al.* *Cell* **58**, 615-622 (1989).
21. Marotta, C. A. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **86**, 337-341 (1989).
22. Robakis, N. K., Ramakrishna, N., Wolfe, G. & Wisniewski, H. M. *Proc. natn. Acad. Sci. U.S.A.* **84**, 4190-4194 (1987).
23. Kunkel, T. A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 488-492 (1988).
24. Brandl, C. J., deLeon, S., Martin, D. R. & MacLennan, D. H. *J. Biol. Chem.* **262**, 3768-3774 (1987).
25. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
26. Sompayrac, L. M. & Danna, K. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7575-7578 (1981).
27. Maruyama, K. & MacLennan, D. H. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3314-3318 (1988).
28. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
29. Springer, T. A. *J. Biol. Chem.* **256**, 3833-3839 (1981).

ACKNOWLEDGEMENTS. We thank N. K. Robakis and H. M. Wisniewski for clone B2.3, T. Ishii for antibodies, F. Kametani for advice, and D. Allsop for critical review of the manuscript. We also thank T. Aizawa for fluorescent microscopy and Y. Izumiya for electron microscopy sample preparation and K. Kato for photographic assistance. This work was supported by the Ministry of Education, Science and Culture of Japan and the Uehara Memorial Foundation.

Structural and serological evidence for a novel mechanism of antigenic variation in foot-and-mouth disease virus

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CHANGES resulting in altered antigenic properties of viruses nearly always occur on their surface and have been attributed to the substitution of residues directly involved in binding antibody. To investigate the mechanism of antigenic variation in foot-and-mouth disease virus (FMDV), variants that escape neutralization by a monoclonal antibody have been compared crystallographically and serologically with parental virus. FMDVs form one of the four genera of the Picornaviridae. The unenveloped icosahedral shell comprises 60 copies each of four structural proteins VP1-4. Representatives from each of the genera have similar overall structure, but differences in the external features¹⁻⁴. For example, human rhinovirus has a pronounced 'canyon' that is proposed to contain the cell attachment site^{1,5-7}, whereas elements of the attachment site for FMDV, which involves the G-H loop (residues 134-160) and C-terminus (200-213) of VP1^{8,9}, are exposed on the surface. Moreover, this G-H loop, which is a major antigenic site of FMDV, forms a prominent, highly accessible protrusion⁴, a feature not seen in other picornaviruses. It is this loop that is perturbed in the variant viruses that we have studied. The amino acid mutations characterizing the variants are not at positions directly involved in antibody binding, but result in far-reaching perturbations of the surface structure of the virus. Thus, this virus

seems to use a novel escape mechanism whereby an induced conformational change in a major antigenic loop destroys the integrity of the epitope.

A neutralizing monoclonal antibody, 24.31, was raised to the O₁ BFS 1860/67 strain, and serological data indicate that its binding is highly sensitive to virus distortion¹⁰. By inhibiting the monoclonal antibody binding to virus with peptides (antigen competition) and anti-peptide sera (antibody competition), components of the epitope have been mapped to VP1 regions 146-150 and 200-213, with the critical involvement of residues 146 and either 206 or 207¹¹. None of the neutralization-resistant variant viruses selected with this antibody have substitutions within either the G-H loop or at the C-terminus. In fact, the six variants with identified mutations all show substitutions within the B-C loop of VP1; at residues 43, 48 and 59. Neither antibody nor antigen competition assays showed any direct involvement of the B-C loop in binding this antibody. Furthermore, anti-(B-C) loop peptide antibodies failed to recognize native virus, although they did react with virus distorted by

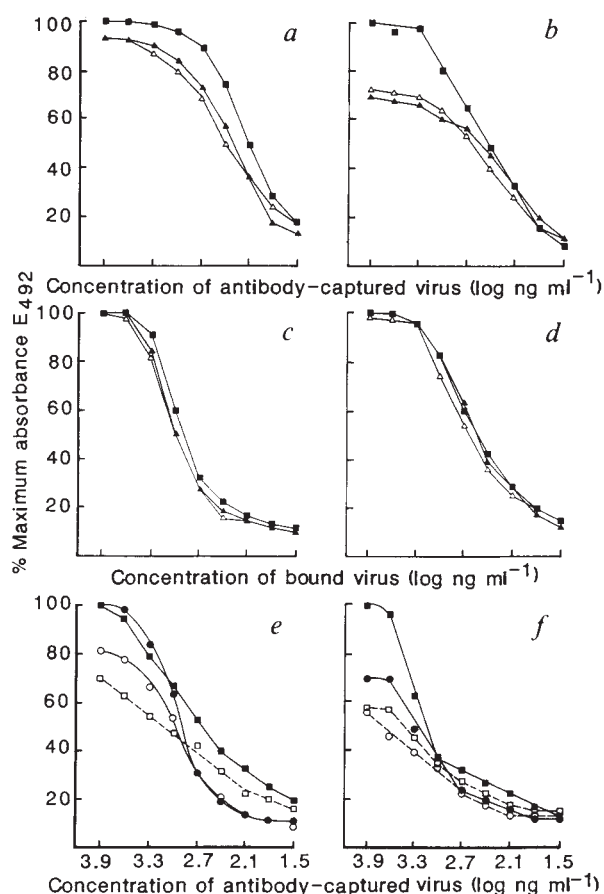
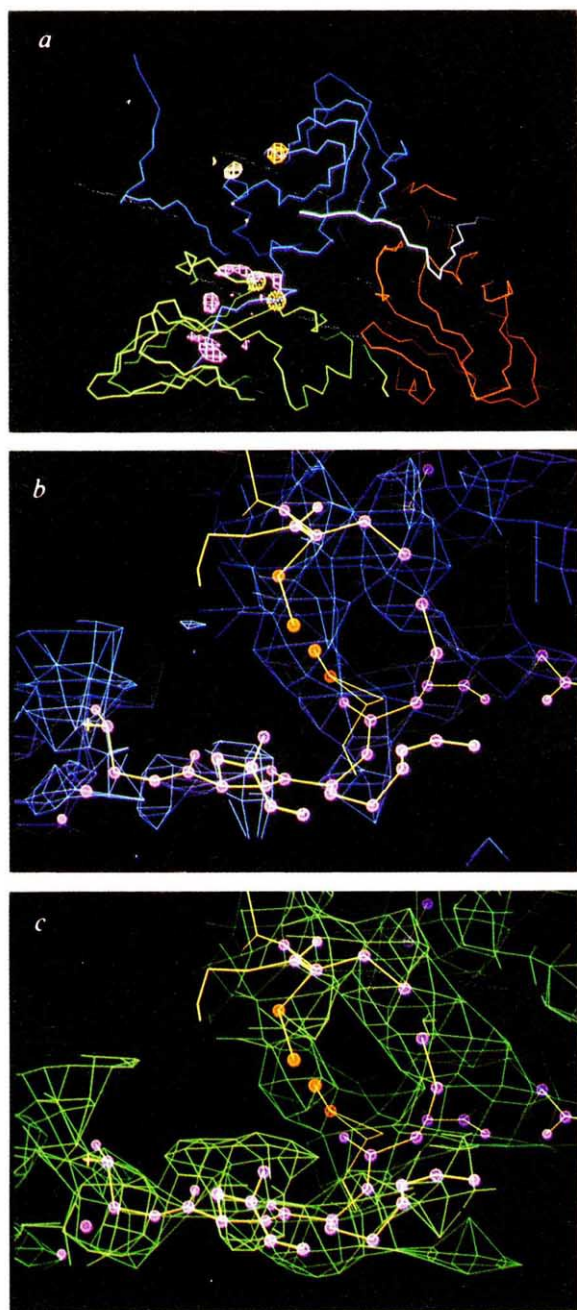


FIG. 1 *a* and *b*, Reactions of parental O₁BFS 1860/67 FMDV (■) and monoclonal antibody escape variants V1 (VP1 Thr 43 → Ala) (△) and V3 (VP1 Thr 43 → Ser) (▲) with anti-virus (*a*) and anti-peptide 141-160 Cys (*b*) antisera in a sandwich ELISA. *c* and *d*, Reactions of the same viruses with antisera to peptides 141-160 Cys (*c*) and 41-60 Cys (*d*) in a direct ELISA. *e* and *f*, Reactions of parental virus (squares) and V4 (circles) (VP1 Ile 48 → Val), serologically indistinguishable from the other variants, with anti-virus (*e*) and anti-peptide 141-160 Cys (*f*) antisera under standard conditions (■, ●) and reducing conditions (□, ○; 0.1% 2-mercaptoethanol) in a sandwich ELISA.

METHODS. In all tests, constant dilutions of antisera were assayed against serial doubling dilutions of virus bound to PVC microplates either with carbonate buffer (direct ELISA) or through an anti-virus IgG capture antibody (sandwich ELISA). The assays were developed using peroxidase-labelled second antibody and peroxide/o-phenylenediamine substrate in citrate buffer.

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trypsin digestion¹² or by binding to plastic^{13,14}. These results suggest that the B-C loop is inaccessible to antibody in the intact O₁ BFS virus.

As the G-H loop had been implicated in the antibody 24.31 epitope, the possibility arose of distinguishing the parent virus from its derived mutants using antisera against G-H loop peptides, even though the viruses have identical sequences in this region. Although the variants reacted as well as the parent virus with anti-virus antisera in sandwich ELISAs (Fig. 1a), the results with anti-peptide antiserum (Fig. 1b) indicated a reduction in the proportion of antibodies binding to the variants. The distinction was lost when the virus was distorted in a direct binding ELISA (Fig. 1c). This finding was corroborated by a 3-4-fold reduction in the ability of the same serum to neutralize the variants compared to the parental strain (data not shown).

These serological data suggest that the substitutions found in the B-C loop are not themselves directly involved in abrogating antibody binding, but induce conformational changes in the G-H loop. To understand the structural basis of these results,

FIG. 2 a, An icosahedral subunit of FMDV; the C α backbone is colour coded: VP1, blue; VP2, green; VP3, red; shown in white is the C terminus of a symmetry-related VP1 (it comes from the five-fold related subunit located to the right). Difference electron density maps for V1 and V3, computed with amplitudes ($F_{\text{obsPAR}} - F_{\text{obsVAR}}$) and α_{averaged} phases, are superimposed in the region of the B-C loop of VP1. The V1 substitution is identified by a clear negative peak at position 43 of VP1 (shown in orange) consistent with the loss of electron density for the Thr $\rightarrow$ Ala sequence change. The V2 substitution, a positive peak, corresponds to a gain in electron density at position 59 of VP1 (shown in lemon). This His $\rightarrow$ Tyr substitution was subsequently confirmed by sequencing. The maps are very clean in the vicinity of these features, reflecting the lack of associated conformational change in the B-C loop region of VP1. Positive difference electron density for V1 is also shown (in pink) corresponding to the switch in population of the disulphide (cysteines marked by yellow, dotted spheres) between position 134 of VP1 and 130 of VP2 with the concomitant movement of strand β -H of VP2 and greater order in the G-H loop of VP1, extending beyond residue 134 in a direction towards the icosahedral threefold axis. The V1 map is representative of the V2 map in this region although of somewhat better quality as the data for V2 are less complete. The difference electron density is contoured at a level of roughly half the maximum peak height in the vicinity of the B-C loop but at a lower level in the region around the G-H loop. Data beyond 4 Å are excluded on account of the error in the weakly measured high angle data; comparable maps result with attenuation of the high angle data. Comparison between these scalar difference maps and vector difference maps²⁶ showed the scalar maps to be more reliable in this case. b and c, Fourier syntheses (with amplitudes ($2F_{\text{obs}} - F_{\text{averaged}}$) and the appropriate α_{averaged} phases) for the parent and variant viruses, respectively, with the V1 model superimposed. The maps show the averaged electron density (corresponding to the five copies of the virus protomer in the I23 asymmetric unit) for both the parent and V1. As we were interested in features on the surface of the virus we performed no solvent flattening. Exactly the same region is depicted in b and c and the maps are contoured at equivalent levels. The atom positions are represented by pink spheres. Note the switch in the dominant conformation for the S-S bond, linking Cys 134 of VP1 and Cys 130 of VP2, between the parent and V1. Alternative Cys side chain atom positions are shown in red. Beyond this, there is increased order in the electron density for V1 (and likewise V2).

we examined two variants V1 (VP1 Thr 43 $\rightarrow$ Ala) and V2 (VP1 His 59 $\rightarrow$ Tyr) by X-ray crystallography. The crystals, grown as described previously¹⁵, were isomorphous with those of the parent virus. Crystallographic details are presented in Table 1. In the present study, individual phases were obtained for each of the data sets by further cycles of averaging¹⁶ against the relevant set of structure factors using iterative real-space averaging and solvent flattening from the set of phases obtained previously⁴. Thus, there was no possibility of bias towards any one of the structures. The appropriate phases were used to calculate a variety of electron density maps. To preserve features on the surface of the virus we performed no solvent flattening on these syntheses (for details see Fig. 2).

The difference electron density maps for both variants show clear evidence for mutations in the B-C loop of VP1 as expected (Fig. 2a). The maps are clean around the substitutions and show no indication of local conformational changes. But differences in electron density are evident at a considerable distance from the mutations and are similar for both variants. We have previously reported bifurcated density for a disulphide bond linking residues 134 of VP1 and 130 of VP2 in the parent virus⁴, which we interpreted as indicating the presence of multiple loop conformations in agreement with the absence of electron density for residues 137-156 of VP1. Of the two conformations, one is predominant in the parent virus, whereas the other predominates in both variants (Fig. 2b, c). This conformational change results in the concomitant movement of strand H of VP2. Furthermore, there is more ordered density in both variants, extending beyond residue 134 of VP1 towards the icosahedral three-fold axis, away from the B-C loop. The 137-142 amino acid sequence of VP1 correlates well with this density and has been built into the electron density map (Fig. 2b, c). Residues 136-139 of VP1 lie on top of strand C at the edge of the β -barrel of VP2 (Fig. 3), forming an extra strand of β sheet and lying in a position

TABLE 1 Summary of X-ray data

	Parent	V1	V2
Number film packs processed	60	46	12
% Completeness to 2.9 Å	69.9	70.4	31.8
RI (all data included)	14.1	10.3	14.9
R	17.84	14.91	16.35
C	0.92	0.94	0.93

All three data sets were derived from a substantial number of crystals of cloned virus. Further, as there is no significant proteolytic degradation of the virus in the crystals¹⁵, we are confident that they contain homogeneous populations of intact particles. The crystals belonged to the cubic space group I23, $a = 345$ Å, with fivefold non-crystallographic redundancy. Data were collected on small angle oscillation photographs with the exception of some supplementary low angle data for V1 collected on the Enraf Nonius FAST electronic area detector. The SERC Synchrotron radiation facility at Daresbury, UK was used throughout, subject to the usual disease security measures. The protocol for the collection of photographic data was as previously described⁴ with the implementation of an automatic indexing procedure²⁵ for analysis of the V2 data. The procedure for the collection and analysis of the FAST data will be described elsewhere.

$$RI = \frac{\sum_h \sum_i |(I_h - I_{hi})|}{\sum_h \sum_i I_{hi}} \times 100$$

$$R = \frac{\sum_h |(|F_{h,obs}| - |F_{h,calc}|)|}{\sum_h |F_{h,obs}|} \times 100$$

$$C = \frac{\sum_h ((F_{h,obs}) - |F_{h,obs}|)(|F_{h,calc}| - |F_{h,calc}|)}{[\sum_h ((F_{h,obs}) - |F_{h,obs}|)^2 \cdot \sum_h ((F_{h,calc}) - |F_{h,calc}|)^2]^{1/2}}$$

I_h is the weighted mean measured intensity of the observations I_{hi} . F_{obs} is the observed structure amplitude of reflection h . F_{calc} is the calculated structure amplitude of reflection h as a result of back-transforming the averaged and modified electron density map.

occupied by part of the 'antigenic puff' of VP2 in the other genera of the picornaviruses, as predicted¹.

In the light of the structural differences observed, the antigenic consequences of reducing the VP1-VP2 disulphide bond were investigated. In the presence of 0.1% 2-mercaptoethanol, which does not affect infectivity, the ability of antiserum to the G-H loop peptide to distinguish between the parent and a variant virus was abolished (Fig. 1e,f). This supports the hypothesis that the disulphide bond between VP1 and VP2 contributes to the antigenic properties of the FMDV loop.

The structural differences provide an explanation of the serological results, pointing to the conclusion that the G-H loop exists in at least two conformations or families of conformation in these viruses: (1) The 'down' conformation, for which there is good structural evidence in the variants, where the G-H loop extends downwards until it approaches close to the pentamer boundary and the icosahedral three-fold axis; (2) the 'up' conformation (postulated to predominate in the parent), where the G-H loop overlays the B-C loop in the region of residues 43 to 59 of VP1. Although there is no conclusive evidence for the 'up' conformation, very weak protein density is seen in association with the B-C loop, consistent with this hypothesis. This is the conformation recognized by antibody 24.31. In the 'down' conformation of the G-H loop, residue 146, a crucial component of the 24.31 epitope is too distant from the B-C loop for direct involvement of the substituted residues in antibody binding (Fig. 3). It seems that the variants escape neutralization through substituted residues in the B-C loop interacting with the G-H loop to destabilize the 'up' conformation, thus destroying the integrity of the epitope recognized by this antibody.

Of the seven FMDV serotypes only type O has the potential to form a VP1-VP2 disulphide bond involving the G-H loop of VP1. The constraints imposed by this bond may be related to the observation that most monoclonal antibodies to serotype O viruses recognize conformationally 'sensitive' epitopes, whereas

a high proportion of monoclonal antibodies to serotype A and C viruses recognize immobilized peptides representing the G-H loop^{17,18}. The conformational restriction of the disulphide bond thus seems to drive the loop towards the less intrinsically stable 'up' conformation. The substitutions in the variant viruses seem to overcome this. Even with viruses lacking the disulphide bond, however, indirect modulation of the conformation of the G-H loop may be important as substitutions in the region of VP2 underlying the 'down' conformation of the loop result in changes in antigenicity and cell attachment¹⁹; properties attributed to the G-H loop itself^{8,9,11,12,20-22}. Although the loop remains too disordered to decipher the detailed mechanism of antibody escape, its dynamic properties may have a significant role in the kinetics of antibody binding as only a small change in the free energy of binding will be needed to reduce dramatically the proportion of antibody molecules that attach.

None of the substitutions in the variant viruses occurred within the previously mapped epitopic regions. As these regions are important for viral attachment to cell receptors⁸, it is possible that the residues crucial for binding of antibody 24.31 are coincident with those necessary for receptor binding and are, therefore, intolerant of mutation. But mutations within the G-H

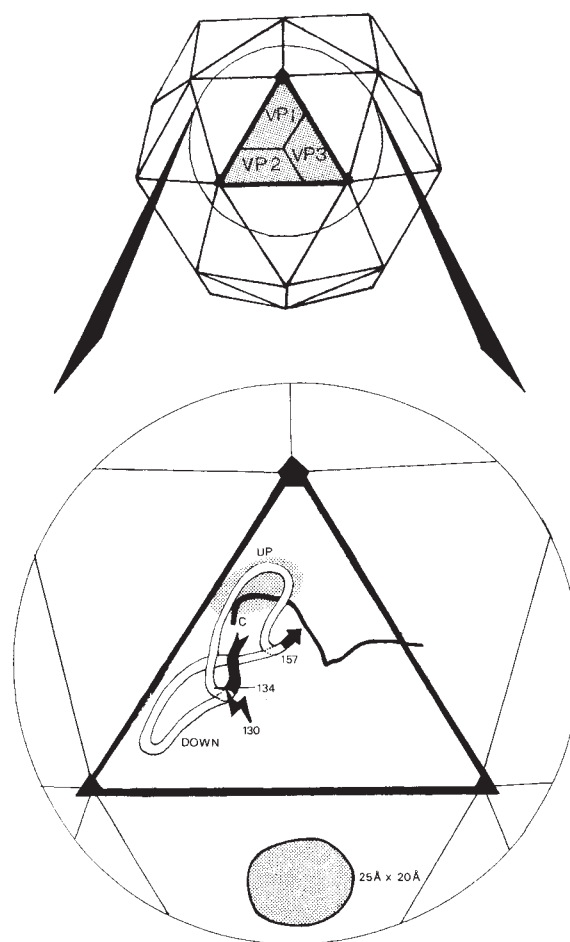


FIG. 3 Schematic diagram of the icosahedral subunit showing the proposed conformational switching of the VP1 G-H loop. The 'down' conformation reflects the extension of the C-terminal end of the FMDV loop as built for V1 running parallel to strand β -C of VP2 before turning down towards the threefold axis and then looping back to the defined location for residue 157. The location of the epitope described involving residues in the G-H loop in the postulated 'up' conformation and the C terminus of VP1 from the adjacent protomer (depicted in black) is stippled. Its location with respect to the disulphide between residue 134 of VP1 and 130 of VP2 can be visualized. An area scaled to represent the approximate area of an antibody 'footprint' is shown.

loop have been selected under pressure from other monoclonal antibodies²². It is known that FMDVs vary in their receptor specificity²³, so different conformations of the G-H loop may be recognized by different receptor molecules. Alternatively, the same receptor may induce conformational changes in the G-H loop on binding.

In summary, we present a model of antigenic variation achieved through a switch in the predominant conformation of a surface loop of FMDV. This change is triggered by substitutions of residues in another surface loop, in a mechanism akin to the allosteric switching of enzymes²⁴. □

Received 28 June; accepted 13 August 1990.

- Rossmann, M. G. *et al. Nature* **317**, 145–153 (1985).
- Hogle, J. M., Chow M. & Filman D. J. *Science* **229**, 1358–1365 (1985).
- Luo, M. *et al. Science* **235**, 182–191 (1987).
- Acharya, R. *et al. Nature* **337**, 709–716 (1989).
- Colonna, R. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 5449–5453 (1988).
- Greve, J. M. *et al. Cell* **56**, 839–847 (1989).
- Staunton, D. E. *et al. Cell* **56**, 849–853 (1989).
- Fox, G. *et al. J. gen. Virol.* **70**, 625–637 (1989).
- Surovoi, A. Y., Ivanov, V. T., Chepurkin, A. V., Ivanyushchenkov, V. N. & Drygalin, N. N. *Soviet J. bio-organic Chem.* **14**, 527–580 (1989).
- Barnett, P. V., Ouldrige, E. J., Rowlands, D. J., Brown, F. & Parry, N. R. *J. gen. Virol.* **70**, 1483–1491 (1989).
- Parry, N. R., Barnett, P. V., Ouldrige, E. J., Rowlands, D. J. & Brown, F. *J. gen. Virol.* **70**, 1493–1503 (1989).
- Strohmaier, K., Franze, R. & Adam, K. H. *J. gen. Virol.* **59**, 295–306 (1982).
- Ouldrige, E. J. *et al. J. gen. Virol.* **65**, 203–207 (1984).
- McCullough, K. C., Crowther, J. R. & Butcher, R. N. *J. immun. Meth.* **82**, 91–100 (1985).
- Fox, G. *et al. J. molec. Biol.* **196**, 591–597 (1987).
- Bricogne, G. *Acta crystallogr.* **A32**, 832–847 (1976).
- Bolwell, C. *et al. J. gen. Virol.* **70**, 59–68 (1989).
- Mateu, M. G. *et al. Virus Research* **8**, 261–274 (1987).
- Bolwell, C. *et al. J. gen. Virol.* **70**, 45–57 (1989).
- Bittle, J. L. *et al. Nature* **298**, 30–33 (1982).
- Thomas, A. A. M., Woortmeijer, R. J., Puijk, W. & Barteling, S. J. *J. Virol.* **62**, 2782–2789 (1988).
- McCahon, D. *et al. J. gen. Virol.* **70**, 639–645 (1989).
- Baxt, B. & Bachrach, H. L. *Virology* **104**, 42–55 (1980).
- Monod, J., Wyman, J. & Changeux, J.-P. *Biochemistry* **5**, 365–385 (1965).
- Kabsch, W. *J. appl. Crystallogr.* **21**, 67–71 (1988).
- Badger, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 3304–3308 (1988).

ACKNOWLEDGEMENTS. We thank the staffs of the Synchrotron Radiation Source, SERC Daresbury Laboratory and Coopers Animal Health Limited for practical assistance; W. Kabsch for his computer program XDS; D. Goodridge for patience over the disease security measures; D. Phillips, G. Taylor and others in the Laboratory of Molecular Biophysics for continuing support. K.R.A. and E.F. were supported by the Medical Research Council, D.L. by the Carnegie Trust; D.S. is a member of the Oxford Centre for Molecular Sciences.

Altered protein conformation on DNA binding by Fos and Jun

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THE protein products of the *c-fos* and *c-jun* proto-oncogenes (Fos and Jun, respectively) form a heterodimeric protein complex that interacts with the activator protein-1 (AP-1) binding site and regulates gene transcription in response to extracellular stimuli¹. Protein dimerization is mediated primarily by a coiled-coil-like structure termed the leucine-zipper and DNA binding occurs primarily through regions of each protein rich in basic amino acids that contact both strands of the AP-1 site^{2–8}. The precise nature of the protein–DNA interaction is unknown as studies concerned with dimerization and DNA binding by Fos and Jun have relied on indirect methods to investigate protein–protein–DNA interactions. Here we have developed assay systems using fluorescence spectroscopy and circular dichroism to monitor dimerization and DNA binding directly. The results indicate that the interaction of Fos and Jun with DNA results in an altered conformation of the protein dimers and an increased α -helical content. These techniques may have general application in studies concerning the

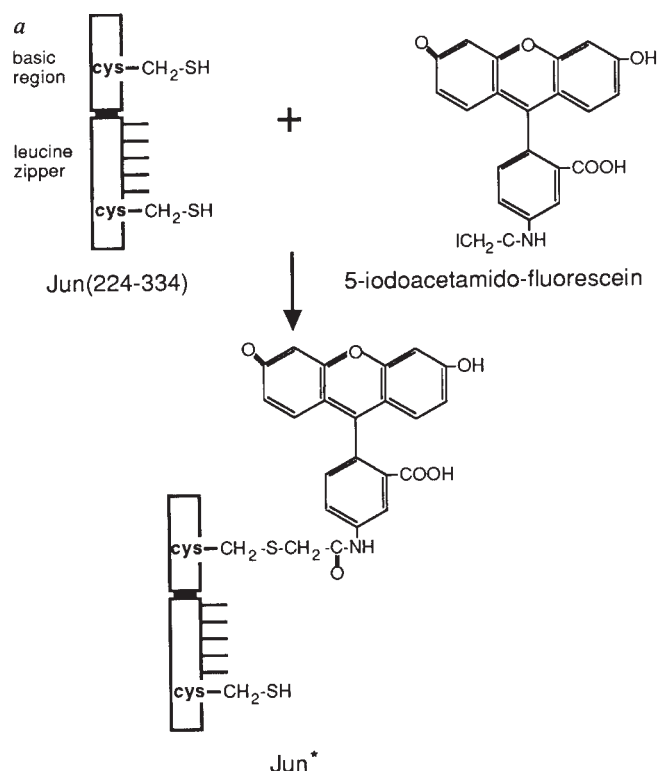
interaction of transcriptional regulatory proteins with specific DNA target sequences.

The sensitivity and specificity of fluorescence spectroscopy techniques have many applications in the analysis of biologically important molecular interactions⁹. For example, fluorescently labelled myosin has been used to investigate the interaction of myosin with nucleotides and actin with great precision¹⁰. We have used a similar approach to develop solution assays for analysing the dimerization and DNA-binding properties of Fos and Jun. The proteins used for these studies contained the leucine-zipper and DNA-binding domains of Fos (amino acids 116–211) and Jun (amino acids 224–334) fused to an N-terminal peptide containing six histidine residues⁸. These proteins were purified from *Escherichia coli* and are active in dimerization, DNA binding and *in vitro* transcription assays²². Fluorescently labelled Jun224–334 was prepared using the thiol-specific reagent 5-iodo-acetamido fluorescein (IAF) (Fig. 1a). There are two potential target cysteine residues for modification in Jun224–334, one located within the DNA-binding domain and one just C-terminal of the leucine zipper¹¹. Under the labelling conditions used, ~1.3 mol of IAF were incorporated per mol of Jun, indicating that each of the Cys residues were labelled. Fluorescently labelled Jun was readily visualized under ultraviolet light after electrophoresis on a sodium dodecyl sulphate polyacrylamide gel (Fig. 1b).

Jun exists primarily as a homodimeric protein complex in solution. The addition of Fos results in an exchange of subunits leading to an accumulation of the more stable Fos–Jun heterodimer^{12–14}. This exchange process was readily observed using IAF-labelled Jun (Jun*). Excitation of Jun* at 490 nm produced a clear emission spectrum with a maximum at 520 nm, which increased substantially after addition of Fos116–211 (Fig. 2a). The increase in fluorescence is likely to be caused by a change in the local microenvironment of the fluorophore. Fluorescein is significantly quenched by acidification and has an increased quantum yield at high pH (ref. 10). Although the emission maximum increased, we did not detect a shift in the wavelength of the excitation or emission spectra. Titration of Jun* with increasing amounts of Fos resulted in a linear elevation of fluorescence (Fig. 2b). The half-maximal increase occurred at a ratio of Jun*:Fos of 1:1 and saturating levels were reached at a ratio of ~1:2 (Fig. 2b). The presence of fluorescent adducts on each partner in the Jun* homodimer may have caused interference resulting in a reduced level of fluorescence of Jun* homodimers compared with Fos–Jun* heterodimers. To test this possibility, we incubated Jun* in the presence of increasing amounts of unlabelled Jun224–334. A mixture of Jun* and unlabelled Jun exhibited increased fluorescence compared with Jun* homodimers (Fig. 2c). As with Fos plus Jun*, the half-maximal increase occurred at a ratio of 1:1. Thus, the exchange of both Jun homodimers and Fos–Jun heterodimers in solution can be monitored by fluorescence spectroscopy.

One of the IAF-labelled Cys residues in Jun224–334 lies within the DNA-binding domain. This is a highly reactive Cys, conserved among all members of the *c-fos* and *c-jun* gene families. Recently, we demonstrated that DNA binding by Fos and Jun is regulated by a reduction/oxidation mechanism at this residue¹⁵. Therefore, we reasoned that DNA binding might cause a sufficient change in the local environment to affect fluorescence. Gel-shift assays demonstrated that the IAF-labelled Jun was capable of binding to DNA (not shown). The presence of a double-stranded oligonucleotide containing an AP-1 binding site (AP-1) caused a significant increase in the emission maximum of a mixture of Fos–Jun* complexes (Fig. 2e). The addition of increasing amounts of the oligonucleotide caused a corresponding increase in fluorescence, although under the conditions used here we did not reach completely saturating levels (Fig. 2f). In contrast, incubation of Jun* or Fos plus Jun* with a nonspecific DNA sequence (deoxyinosine/deoxycytidine, poly(dIdC)) or with an oligonucleotide containing an SP-1 site

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had no significant effect (data not shown). We have also analysed mutated Jun proteins that contain serine substituted for the Cys residues¹⁵. After incubation with IAF, Jun with no Cys was not labelled, Jun with Cys only in the DNA-binding domain was labelled very efficiently and Jun with Cys in the C-terminal region was labelled inefficiently. Thus, it is likely that the modified Jun studied here contains the fluorophore primarily on the highly reactive Cys in the DNA-binding domain. The changes in fluorescence that occurred during dimerization or DNA binding were only seen with the mutated Jun containing the fluorophore in the DNA-binding domain. Thus, DNA binding by Jun* results in a change in the fluorophore microenvironment in the DNA-binding domain although the nature of the change is not clear. It is unlikely that fluorescein binds to DNA

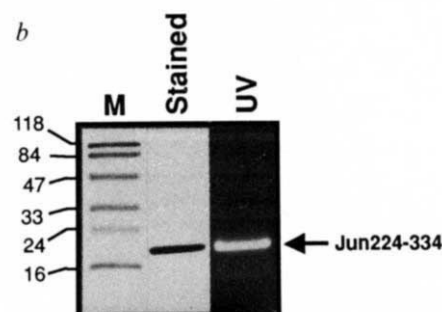
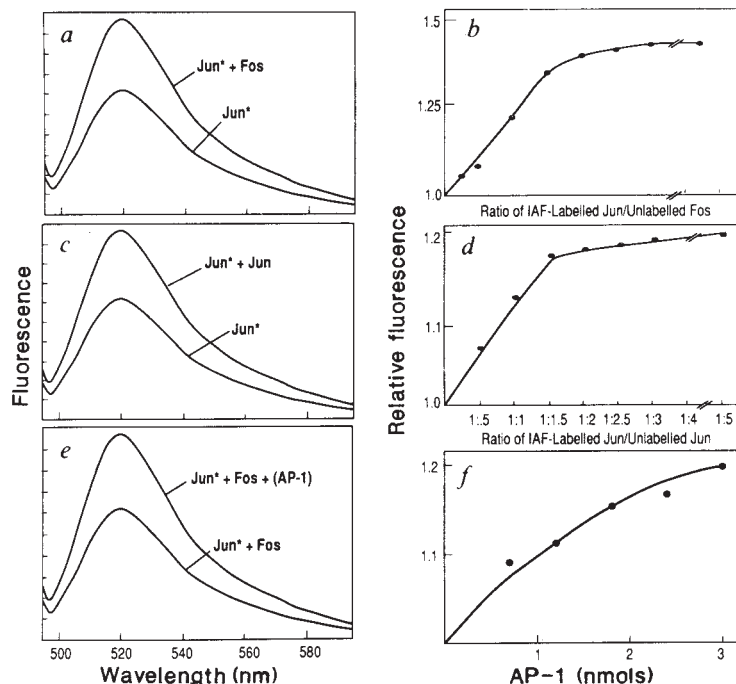


FIG. 1 Fluorescence labelling of Jun. **a**, Jun224-334 was incubated with 2 mM IAF in the presence of 50 mM sodium phosphate (pH 7.5) and 10% glycerol (buffer A) at 4 °C for 16 h in the dark. Untreated fluorophore was removed by extensive dialysis against buffer A supplemented with 10 mM dithiothreitol (DTT). The fluorescein analogue was cross-linked to two Cys residues, one in the DNA-binding domain and one in the C-terminal region of the leucine-zipper. For clarity only one potential product is shown. **b**, Unlabelled Jun224-334 and IAF-labelled Jun were analysed by electrophoresis on a 12% SDS polyacrylamide gel. The unmodified protein was visualized by staining with Coomassie brilliant blue and IAF-labelled protein by illumination with ultraviolet light (UV). Lane M contained molecular weight standards, relative molecular mass (M_r) indicated on left-hand side (K).

as no change in quantum yield was detected in the absence of Jun and the effect was specific for the AP-1 site. It is possible that DNA induces a conformation change in Jun that exposes the fluorophore to the highly basic DNA-binding domain. Mutagenesis studies and experiments with sulphhydryl-modifying agents suggest that the Cys in the DNA-binding domain is in close proximity to DNA¹⁵. We are currently using different fluorophores on each Cys residue in both partners of the heterodimeric complex, and modified bases in the target DNA sequence in an attempt to obtain estimates of the distances between each group in solution.

It has been suggested that the DNA-binding regions of Fos and Jun participate in a 'scissors-grip' interaction with DNA in which α -helical regions lie along the major groove¹⁶. In this model, it is unlikely that the DNA-binding domains would maintain a stable configuration in the absence of DNA. The DNA-binding domains of Fos and Jun are adjacent to the

FIG. 2 Analysis of dimerization and DNA-binding properties of Fos and Jun by fluorescence spectroscopy. The emission spectrum of 1 μ M IAF-labelled Jun (Jun*) in buffer A was recorded at 27 °C between 500–600 nm using a Perkin-Elmer MPF66 fluorometer. The spectra of: **a**, Jun* alone, Jun* plus 5 μ M Fos116–211; **c**, Jun* alone, Jun plus 5 μ M unlabelled Jun224–334; and **e**, 2 μ M Jun* plus 5 μ M Fos in the presence and absence of 3 μ M of a specific double-stranded oligonucleotide containing an AP-1 site (AP-1) (ref. 12) were determined. The effects of increasing amounts of: **b**, Fos 1–5 μ M; **d**, Jun 1–5 μ M; and **f**, 1–3 μ M of the AP-1 oligonucleotide on the emission maximum of Jun* were also examined.



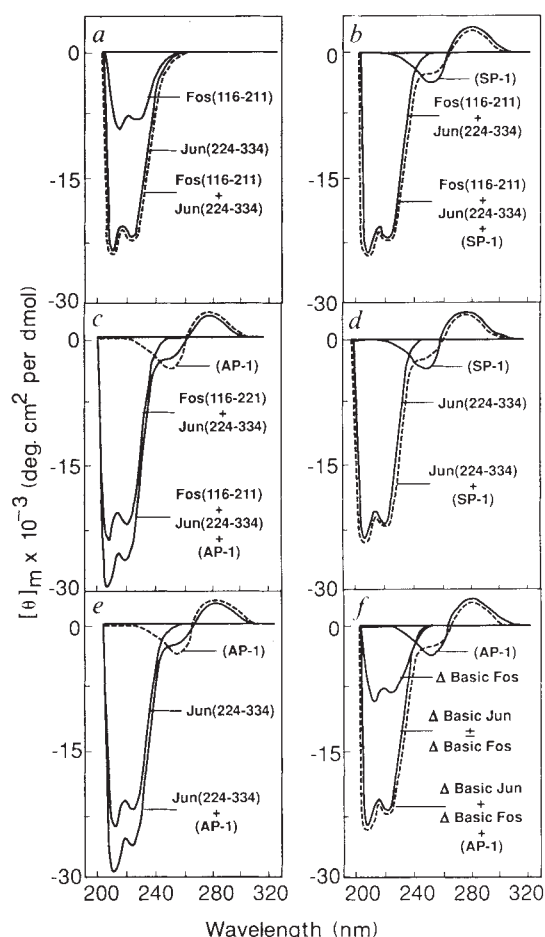


FIG. 3 Effects of dimerization and DNA-binding on CD spectra of Fos and Jun. Polypeptides corresponding to Fos116–211 (Fos) and Jun224–334 (Jun) were analysed in buffer A containing 1 mM DTT at a final protein concentration of 0.2 mg ml⁻¹. (Identical results were obtained using buffer A without glycerol.) Mixtures of proteins and nucleic acids were incubated at 37 °C for 30 min and spectra were recorded for 15 min at 25 °C using a Jasco J500 spectrometer. Representative traces are shown of the spectra of: *a*, Fos, Jun, equimolar mixtures of Fos plus Jun and the reference spectrum of buffer A; *b*, Fos plus Jun in the presence and absence of 0.1 mg ml⁻¹ of a double-stranded oligonucleotide containing an SP-1 site (SP-1) and the SP-1 oligonucleotide alone; *c*, Equimolar mixtures of Fos plus Jun in the presence and absence of 0.1 mg ml⁻¹ of a double-stranded oligonucleotide containing an AP-1 site (AP-1) and the AP-1 oligonucleotide alone; *d*, Jun in the presence and absence of 0.1 mg ml⁻¹ of an oligonucleotide containing an SP-1 site and the SP-1 oligonucleotide alone; *e*, Jun in the presence and absence of 0.1 mg ml⁻¹ of an oligonucleotide containing an AP-1 site and the AP-1 oligonucleotide alone; *f*, analysis of mutated Fos and Jun proteins lacking the DNA-binding region (Δ Basic) in the presence and absence of 0.1 mg ml⁻¹ of an oligonucleotide containing the AP-1 site and the AP-1 oligonucleotide alone. Δ Basic Fos is Fos116–211 lacking amino acids 139–144 and Δ Basic Jun is Jun 224–334 lacking amino acids 260–266. These proteins do not bind to DNA³. The spectra obtained with Δ Basic Jun alone and a mixture of Δ Basic Jun plus Δ Basic Fos were identical and are referred to as Δ Basic Jun $\pm$ Δ Basic Fos. Spectra that overlap are indicated by broken lines. The percentage helicity was calculated as described²¹.

leucine-zipper region, which has been proposed to form a stable α helix that participates in a coiled-coil interaction^{3,17}. Circular dichroism (CD) and two-dimensional nuclear magnetic resonance studies on synthetic leucine-zipper peptides have supported this contention^{14,18}. We analysed the CD spectra of purified Fos116–211 and Jun224–334 to determine their overall α -helical content. Jun224–334 gave a clear spectrum with minima at 208 and 222 nm indicating an α -helical content of ~50% (Fig. 3*a*). In contrast, Fos116–211 gave a relatively weak spectrum indicating a lower α -helical content of ~23%. The simplest explanation

of these data is that Jun forms homodimers that stabilize the α -helical region of the leucine-zipper; whereas Fos does not. This would predict that in a mixture of Fos plus Jun, which form heterodimers, the α -helical content would be similar to that of Jun homodimers. Indeed, this was the case; equimolar mixtures of Fos plus Jun gave a spectrum identical to that of Jun homodimers (Fig. 3*a*). Thus, Fos has a conformation in a Fos–Jun heterodimer substantially different from that of the free Fos monomer in solution.

To examine the effects of DNA on α -helical content, the CD spectrum of Fos–Jun heterodimers was analysed in the presence of an oligonucleotide containing an SP-1 site (Fig. 3*b*) or AP-1 site (Fig. 3*c*). The presence of the AP-1 site caused a distinct increase in the α -helical content of the protein complex of ~10%; whereas the SP-1 site had no significant effect (Fig. 3*b*, *c*). Similar observations were obtained with Jun homodimers (Fig. 3*d*, *e*). The effect of the AP-1 site on conformation was concentration-dependent and saturation levels were reached at a 1:1 ratio of protein:DNA. These results indicate that binding of Fos–Jun heterodimers and Jun homodimers to a specific DNA target sequence, the AP-1 site, causes a change in conformation that results in increased α helicity. This effect may be interpreted in two ways: (1) DNA binding stabilizes the conformation of protein dimers resulting in an overall increase in α -helical content, or (2) the DNA-binding domains of Fos and Jun take on an α -helical configuration only in the presence of DNA. We favour the latter interpretation as Jun homodimers are less stable than Fos–Jun heterodimers, yet they have identical CD spectra (Fig. 3*a*). Similarly, CD studies on synthetic leucine-zipper peptides revealed that per cent helicity, unlike the T_m , was not affected by the stability of the dimers¹⁴. Furthermore, mutated Fos and Jun proteins lacking functional DNA-binding domains (Δ Basic Fos and Δ Basic Jun) failed to show increased α helicity in the presence of the AP-1 site, although Δ Basic Fos showed an increased α helicity when dimerized with Δ Basic Jun (Fig. 3*f*). These data are consistent with the 'scissor-grip' model of DNA binding by proteins containing a leucine-zipper(s)¹⁶. But they neither confirm nor deny the proposed helix-bend-helix structure.

The finding that the DNA-binding domains of Fos and Jun have different configurations depending on whether or not they are associated with DNA has an interesting implication. This would provide a mechanism whereby non-DNA-bound (inactive) and DNA-bound (active) transcription factor complexes might be distinguished. This could be an important feature of transcriptional regulation as non-DNA-bound complexes could sequester components of the transcriptional machinery by the phenomenon of squelching¹⁹. In Fos and Jun, the activation regions of each protein which are responsible for transcriptional stimulation are located very close to the DNA-binding domains^{20,22}. Thus, the conformation of these regions is likely to be altered depending on the state of the DNA-binding domain (that is, whether or not it is associated with DNA). Thus, conformational changes of transcription factors in the presence of specific DNA-binding sites could represent a novel mechanism for regulation. □

Received 18 May; accepted 15 August 1990.

- Curran, T. & Franza, B. R. Jr *Cell* **55**, 395–397 (1988).
- Kouzarides, T. & Ziff, E. *Nature* **336**, 646–651 (1988).
- Gentz, R., Rauscher, F. J. III, Abate, C. & Curran, T. *Science* **243**, 1695–1699 (1989).
- Turner, R. & Tjian, R. *Science* **243**, 1689–1694 (1989).
- Ransone, L. J., Sassone-Corsi, P. & Verma, I. M. *Genes Dev.* **3**, 770–781 (1989).
- Schuerman, M. et al. *Cell* **56**, 507–516 (1989).
- Cohen, D. R. & Curran, T. *Oncogene* **5**, 929–939 (1990).
- Abate, C. et al. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1032–1036 (1990).
- Lakowicz, J. R. in *Principles of Fluorescence Spectroscopy* (Plenum, New York, 1972).
- Aguirre, R., Gonsoulin, F. & Cheung, H. C. *Biochemistry* **25**, 6827–6835 (1986).
- Bohmann, D. et al. *Science* **238**, 1386–1392 (1987).
- Rauscher, F. J. III, Voulalas, P. J., Franza, B. R. Jr. & Curran, T. *Genes Dev.* **2**, 1687–1699 (1988).
- Nakabeppu, Y., Ryder, K. & Nathans, D. *Cell* **55**, 907–915 (1988).
- O'Shea, E. K., Rutkowski, R., Stafford, W. F. III & Kim, P. S. *Science* **245**, 646–648 (1989).
- Abate, C., Patel, L., Rauscher, F. J. III & Curran, T. *Science* (in the press).

16. Vinson, C. R., Sigler, P. B. & McKnight, S. L. *Science* **246**, 911–916 (1989).
17. O'Shea, E. K., Rutkowski, R. & Kim, P. S. *Science* **243**, 538–542 (1989).
18. Oas, T. G., McIntosh, L. P., O'Shea, E. K., Dalquist, F. W. & Kim, P. S. *Biochemistry* **29**, 2891–2894 (1990).
19. Ptashne, M. *Nature* **335**, 683–689 (1989).
20. Bohmann, D. & Tijan, R. *Cell* **59**, 709–717 (1989).
21. Brovotto-Cruz, J. & Li, C. H. *Biochemistry* **8**, 4701–4708 (1969).
22. Abate, C., Luk, D., Gagne, E., Roeder, R. & Curran, T. *Mol. Cell biology* (in the press).

ACKNOWLEDGEMENTS. We thank P. Roepe for advice on fluorescence spectroscopy and M. Boublik for advice on circular dichroism.

Folding transition in the DNA-binding domain of GCN4 on specific binding to DNA

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PROTEIN-DNA recognition is often mediated by a small domain containing a recognizable structural motif, such as the helix–turn–helix¹ or the zinc-finger². These motifs are compact structures that dock against the DNA double helix. Another DNA recognition motif, found in a highly conserved family of eukaryotic transcription factors including C/EPB, Fos, Jun and CREB, consists of a coiled-coil dimerization element—the leucine-zipper—and an adjoining basic region which mediates DNA binding³. Here we describe circular dichroism and ¹H-NMR spectroscopic studies of another family member, the yeast transcriptional activator GCN4^{4,5}. The 58-residue DNA-binding domain of GCN4, GCN4-p, exhibits a concentration-dependent α -helical transition, in accord with previous studies of the dimerization properties of an isolated leucine-zipper peptide⁶. The GCN4-p dimer is ~70% helical at 25 °C, implying that the basic region adjacent to the leucine zipper is largely unstructured in the absence of DNA. Strikingly, addition of DNA containing a GCN4 binding site (AP-1 site) increases the α -helix content of GCN4-p to at least 95%. Thus, the basic region acquires substantial α -helical structure when it binds to DNA. A similar folding transition is observed on GCN4-p binding to the related ATF/CREB site, which contains an additional central base pair. The accommodation of DNA target sites of different lengths clearly requires some flexibility in the GCN4 binding domain, despite its high α -helix content. Our results indicate that the GCN4 basic region is significantly unfolded at 25 °C and that its folded, α -helical conformation is stabilized by binding to DNA.

The DNA-binding domain of the yeast transcription factor GCN4, located within the C-terminal 60 residues of the protein⁷, can be divided into two subdomains. The C-terminal subdomain (32 residues) contains four leucine residues in a heptad repeat, the leucine zipper. Physicochemical studies of a synthetic leucine-zipper peptide^{6,8} and 'zipper swap' experiments^{9,10} support the proposal¹¹ that the leucine repeat forms a hydrophobic dimerization interface. The N-terminal subdomain (the basic region; 26 residues) is rich in lysine and arginine residues, many of which are conserved in this family of transcription factors. Domain swap experiments demonstrate that the basic region has the predominant role in making specific DNA contacts¹².

To further our understanding of the structural basis for DNA recognition by this family of transcription factors, we have examined by circular dichroism (CD) and ¹H-NMR spectroscopy, the structure of a peptide (GCN4-p; residues 225–281)

that includes the basic region and leucine repeat of GCN4. GCN4-p was overexpressed in *Escherichia coli*, and its DNA-binding properties were analysed by gel-retardation assay (Fig. 1). GCN4-p binds to synthetic DNA containing a consensus AP-1 target site with an affinity ($K_{\text{apparent}} = 2 \times 10^{-8}$ M; Fig. 1, lanes 1–6) comparable to that of intact GCN4 (ref. 4). GCN4-p fails to bind to an unrelated DNA sequence (the λ O_L1 operator; Fig. 1, lanes 13 and 14). These results show that the DNA-binding properties of the GCN4-p peptide are very similar to those of the intact GCN4 protein.

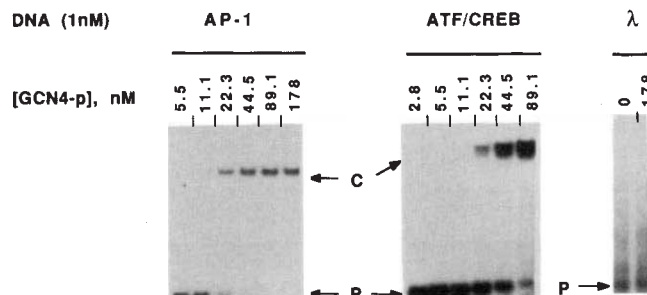
At high protein concentrations (>100 μ M) the far-ultraviolet CD spectrum of GCN4-p shows the characteristics of α -helical structure (Fig. 2A). The calculated α -helix content of GCN4-p under these conditions is 70–73%, or 40–43 residues in helical conformation, calculated on a mean residue ellipticity at 222 nm and 25 °C of $-22,700 \text{ deg cm}^2 \text{ dmol}^{-1}$. No increase in helix content is seen at higher protein concentrations. Progressive attenuation of the α -helix-associated absorption bands is observed with decreasing peptide concentration (Fig. 2A), indicating an unfolding transition that is presumably accompanied by dissociation of the dimer into unstructured monomers. Previous studies of a leucine-zipper peptide (residues 252–281⁶) indicated that it is dimeric throughout the concentration range studied here. We suggest that decreased stability of GCN4-p dimers relative to those of the leucine-zipper peptide may result from repulsion of the basic region subdomains.

The unfolding of GCN4-p with decreasing concentration is also seen by ¹H-NMR spectroscopy. The leucine-zipper subdomain contains two aromatic residues, Tyr 265 and His 266, whose resonances are well resolved and serve as intrinsic probes of GCN4-p dimerization (Fig. 2B). At high and low peptide concentrations (Fig. 2B; spectra a and f, respectively), the resonance linewidths ($1/T_2$) of these residues are consistent with the presence of dimeric and monomeric species. Higher-order oligomers would be expected to show broader resonances, which are not observed. At intermediate peptide concentrations, the pairs of aromatic NMR resonances corresponding to monomeric and dimeric species have relative intensities that vary with GCN4-p concentration (Fig. 2B, spectra b–e). By contrast, NMR resonances of basic region residues (for example, Thr 236) show no intensity change over this range of peptide concentration (data not shown). These results unambiguously assign the concentration-dependent increase in α -helix content of GCN4-p (Fig. 2A) to the leucine-zipper region of the peptide.

Previous studies of the dimerization properties of an isolated leucine-zipper peptide showed that the orientation of α -helices in the dimer is parallel, and it was proposed that the leucine zipper is a coiled-coil structure⁶. Two-dimensional NMR studies of this peptide shows that it forms a continuous α -helix, with symmetrical dimerization contacts that are evident from the single resonance observed for each proton in the peptide⁸. This symmetry is consistent with the parallel association of peptide α helices in a coiled-coil arrangement. The dimer-related protons of GCN4-p also show a single resonance (for example, His 265 and His 265'; Fig. 2Ba), suggesting that the dimerization interface of the GCN4-p DNA binding domain is similar to that observed for the leucine-zipper peptide. The estimated lifetime of GCN4-p dimers at 25 °C is between 10 ms and 1 s, on the basis of the exchange properties of NMR resonances assigned to the leucine-zipper region (see Fig. 2). In conjunction with the modest dissociation constant of GCN4-p for DNA, these observations suggest that unfolding and reassembly of GCN4, and other transcription factors utilizing a coiled-coil dimerization interface, may occur without significant kinetic barriers, thereby facilitating subunit exchange. Such exchange is thought to provide a general mechanism for regulating patterns of gene expression.

Our results indicate that the leucine zipper dimerization interface is present in a functional GCN4 DNA-binding domain; but the α -helix content of GCN4-p (40–43 residues) is greater

FIG. 1 Gel-retardation assay of GCN4-p binding to oligonucleotides containing the AP-1/TPA-responsive element consensus binding site (5'-GAGAT-GAGTCATCTC-3'; lanes 1-6), the activating transcription factor/cyclic AMP-responsive element consensus binding site (5'-GAGATGACGTCATCTC; lanes 7-12), or the phage λ O₁ site (5'-GATACCACTGGCGGTGATATC-3'; lanes 13 and 14). 5'-³²P-labelled oligonucleotide (1 nM) was incubated with the indicated concentrations of GCN4-p peptide for 30 min at 23 °C in buffer containing 50 mM KPO₄ (pH 7.5), 50 mM KCl, 100 μ g ml⁻¹ BSA and 5% glycerol then electrophoresed on a 5% polyacrylamide gel. Following electrophoresis, gels were dried and the free oligonucleotide probe (P) and GCN4-p-DNA complexes (C) were visualized by autoradiography. The GCN4-p peptide (residues 225-281 of the GCN4 protein) was expressed in *E. coli* from a bacteriophage T7 RNA polymerase promoter^{24,25} (10-12 mg peptide per litre culture) and purified by a combination of ammonium sulphate precipitation and phosphocellulose chromatography as described elsewhere to >98% homogeneity as judged by SDS-PAGE. An extinction coefficient of 1,313 M⁻¹ cm⁻¹ at 280 nm, derived by amino acid analysis and ultraviolet



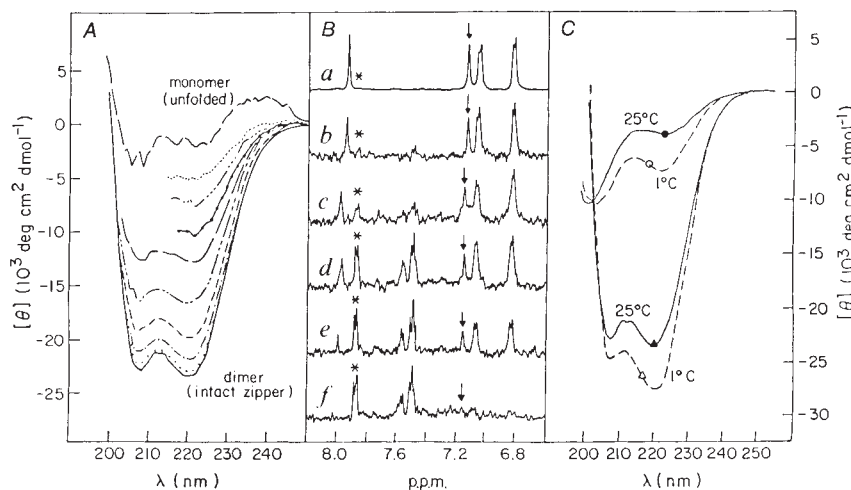
spectrophotometry, was used to determine the concentration of GCN4-p monomer in the stock solution. Oligonucleotides used in all assays were synthesized on a Milligen or an Applied Biosystems oligonucleotide synthesizer and purified by HPLC or gel electrophoresis.

than can be accounted for by the leucine-zipper subdomain alone (32-36 residues). Indeed, the CD spectrum of GCN4-p exhibits more α helix as the temperature is lowered. At 1 °C, GCN4-p is ~80% helical, as calculated from a mean residue ellipticity of -27,000 deg cm² dmol⁻¹ under these conditions (Fig. 2C). We propose that the basic region of GCN4-p exists as an ensemble of structures in the absence of DNA, with a substantial population of completely folded molecules present only at low temperature. Support for this proposal comes from studies of a synthetic basic region peptide (GCN4-br; residues 224-249), which exhibits, between 25 °C and 1 °C, a partial α -helical transition similar in magnitude to that of GCN4-p over the same temperature range (Fig. 2C). The thermal unfolding transitions of GCN4-p are described in detail elsewhere¹³.

The CD spectrum of the specific complex between GCN4-p and a synthetic AP-1 site (21 base pairs) is shown in Fig. 3A. A significant increase in the magnitude of the helix-associated bands at 205 and 225 nm is observed for the complex (Fig. 3A),

relative to GCN4-p alone (Fig. 2A). This change is opposite to that expected from the contribution of DNA, which exhibits positive ellipticity in this region of the spectrum (Fig. 2A, upper spectrum). The spectral perturbations resulting from complex formation are therefore the result of a DNA-dependent structural transition in the protein, as evidenced by the difference spectrum of the complex minus the sum of its constituents (Fig. 3A, Δ). The magnitude of this difference spectrum increases linearly as a function of added AP-1 binding site, reaching saturation at a stoichiometry of two GCN4-p monomers per DNA site (data not shown). At saturation, the DNA-bound conformation of GCN4-p is 95-100% α -helix (Fig. 3A). Thus, our results indicate that the folded conformation of the GCN4-p basic region is predominately α -helical, but that the folded state is significantly populated only at low temperature (Fig. 2C) or on binding to DNA (Fig. 3C). The helical transition observed for the isolated basic region peptide at low temperature (Fig. 2C) suggests that these conformational properties of the GCN4

FIG. 2 A, normalized far-ultraviolet CD spectra of GCN4-p at different protein concentrations in 200 mM KCl, 50 mM potassium phosphate (pH 7.0 and 25 °C). Protein concentrations (bottom to top, μ M): 155, 38, 13, 4.3, 1.6, 1.2, 0.9, 0.6, 0.3, 0.15. Gel-filtration chromatography demonstrates that GCN4-p is dimeric at a protein concentration of 2 mM under these conditions¹³. The GCN4-p monomer seems to be unfolded, as has previously been observed among certain prokaryotic DNA-binding proteins²⁶. To maintain adequate signal-to-noise at lower protein concentrations cuvettes with longer path lengths were used (range: 0.5-10 mm). Protein concentration was determined by quantitative amino acid analysis following acid hydrolysis of an aliquot. Mean residue ellipticity $[\theta]$ was calculated with calculated molecular weight 6,874 g mol⁻¹ (118.5 g per mol-residue). B, Aromatic region of the 500 MHz ¹H-NMR spectrum of GCN4-p at different protein concentrations in 500 mM KCl, 50 mM potassium phosphate (pD 7.0, direct meter reading) in 99.98% D₂O. Fortuitously, the apparent dimerization constant of GCN4-p is weaker under these conditions, facilitating NMR study. a-f, 1,000, 100, 50, 30, 20 and 10 μ M, respectively. Dimer-specific and monomer-specific aromatic resonances are observed in slow exchange on the NMR time scale. Arrow indicates dimer-specific H δ resonance of H266, which decreases in amplitude as the protein concentration is decreased. *, monomer-specific aromatic resonance of Y265, which increases in amplitude as the protein concentration is decreased. The slow-exchange condition provides a lower bound of 10 ms on the lifetime of the dimer. The unresolved resonances of the branched-chain aliphatic sidechains (leucine, isoleucine and valine), which are primarily in the leucine-zipper moiety, are in intermediate exchange under these conditions (data not shown), providing an upper bound of 1 s on the lifetime of the dimer. This short lifetime of the GCN4-p dimer is in apparent conflict with the previous observation suggesting that heterodimers between intact GCN4 and the C-terminal domain were very stable at 0-4 °C in the absence of DNA⁵. But recent experiments suggest



that the original observations were artifactual, probably because of co-precipitation of the proteins with excess nonspecific nucleic acid in the particular wheat germ extracts employed at that time. The chemical shift scale was referenced to TSP resonance at 0 p.p.m. C, Normalized CD spectra of the GCN4-p dimer (Δ) and the basic-region peptide ($\circ$) at 25 °C and 1 °C in the buffer described in (A). The concentration of GCN4-p was 145 μ M; the concentration of the basic-region peptide was 154 μ M. The sequence of the basic-region peptide is (N)SSDPAALKRARNTAARRSRARKLQR-CONH₂ (single letter amino-acid code). Its structure was verified by amino-acid analysis and sequencing; no truncation or deletion products were observed. The helix content is estimated from the mean residue ellipticity at 222 nm, assuming that for a 100% helical peptide this value is -33,000 deg cm² dmol⁻¹ at 0 °C, and at higher temperatures is attenuated 0.3% per °C before unfolding^{6,27-29}. GCN4-p exhibits a cooperative unfolding transition (midpoint 65 °C) similar to that observed in the isolated GCN4 leucine-zipper peptide²⁷, as will be described elsewhere¹³.

basic region are locally determined and are independent of the adjoining leucine-zipper dimerization interface.

Two sets of experiments demonstrate that the folding transition observed on addition of the AP-1 DNA to GCN4-p requires specific complex formation. First, the spectral changes do not occur at high ionic strength (Fig. 3A). The CD spectrum of the GCN4-p/AP-1 mixture in 1.5 M KCl is nearly identical to that of GCN4-p alone. The CD spectrum of GCN4-p alone is unaltered in 50–1,500 mM KCl (not shown). Moreover, the KCl-dependence of the DNA-induced spectral changes is consistent with the elution properties of AP-1 site-containing oligonucleotides from a GCN4 affinity column¹⁴. Second, the CD spectrum of a mixture of GCN4-p with a heterologous DNA control element that does not bind to GCN4-p (the λ *O*_L1 operator site, Fig. 1) resembles the sum of the spectra of the constituents alone (Fig. 3B). The calculated difference spectrum for this mixture (Fig. 3B, Δ) has less than 10% of the magnitude of the GCN4-p/AP-1 difference spectrum (Fig. 2A). Interestingly, the small perturbation induced by the nonspecific DNA site is consistent with partial stabilization of α -helix.

The DNA-dependent folding of GCN4-p was further examined using a consensus ATF/CREB binding site¹⁵, which differs from the AP-1 site only in the addition of a central CG base pair. In B-form DNA, the two half-sites of this longer element are displaced by a translation of about 3.4 Å and a rotation of 34° relative to their position in the AP-1 site. Although the AP-1 and ATF/CREB sites seem to be targets for distinct families of mammalian leucine-zipper proteins¹⁵, GCN4-p binds to both sites with comparable affinity (Fig. 1). Correspondingly, CD studies of the complex of GCN4-p with an oligonucleotide containing the ATF/CREB site show a folding transition similar to that observed on binding of GCN4-p to the AP-1 sequence (Fig. 3C). The CD spectra of both the AP-1 and ATF/CREB DNAs in the region of 250–300 nm are consistent with B-form structure (Fig. 3D). Similar, small perturbations in each spectrum are observed on binding GCN4-p. We conclude that the alternative half-site spacings are accommodated by flexibility in GCN4-p rather than by major structural rearrangements in the DNA. Binding to target sites of different length is also observed for *lac* repressor¹⁶, where it is attributed to flexible tethering of individual *lac* headpieces to the core tetramer^{17–19}.

Our observations by CD spectroscopy of an α -helical folding transition in GCN4-p lead to the following conclusions: (1) the complete GCN4 DNA binding domain undergoes the same dimerization-dependent coil-to-helix transition seen with an isolated leucine-zipper peptide; (2) the basic region of GCN4-p undergoes a coil-to-helix transition when it binds to DNA; (3) α -helical conformation can also be induced in the GCN4-p basic region by lowering the temperature; (4) similar conformational changes are induced in the protein on binding to sites with AP-1 or ATF/CREB consensus elements, implying flexibility in the link between the basic region and the leucine-zipper.

Can our conclusions concerning the coil-to-helix change we have observed when GCN4-p binds DNA be extended to the intact GCN4 protein? We argue strongly that the basic region of intact GCN4 also undergoes such a DNA-dependent folding transition, on the basis of the following observations: (1) GCN4-p binds specifically, with an affinity comparable to that of intact GCN4. Were the basic region of the intact protein 'prefolded,' for example by interaction with segments not present in GCN4-p, its DNA affinity should be higher, corresponding to the free energy expended in folding the peptide; (2) a 60-residue species essentially equivalent to GCN4-p, produced in an *in vitro* translation experiment, binds as a homodimer to a HIS3 target site with the same affinity as a variety of a larger species⁴; (3) in experiments where this 60-residue segment is present with longer chains, large homodimers, heterodimers, and small (60mer) homodimers all bind with the same affinity⁵; (4) our picture of coupled folding and binding is fully consistent with the implications of chemical modification experiments,

which show GCN4 and related proteins make extensive contacts with their cognate binding sites^{20–22}.

These results imply that some part of the DNA-binding domain must be loosely structured to 'wrap' around the DNA helix. One precedent for flexibility in a DNA-binding domain is the N-terminal arm of the λ repressor, which is disordered in the free protein²³. The arm binds to the 'back side' of the

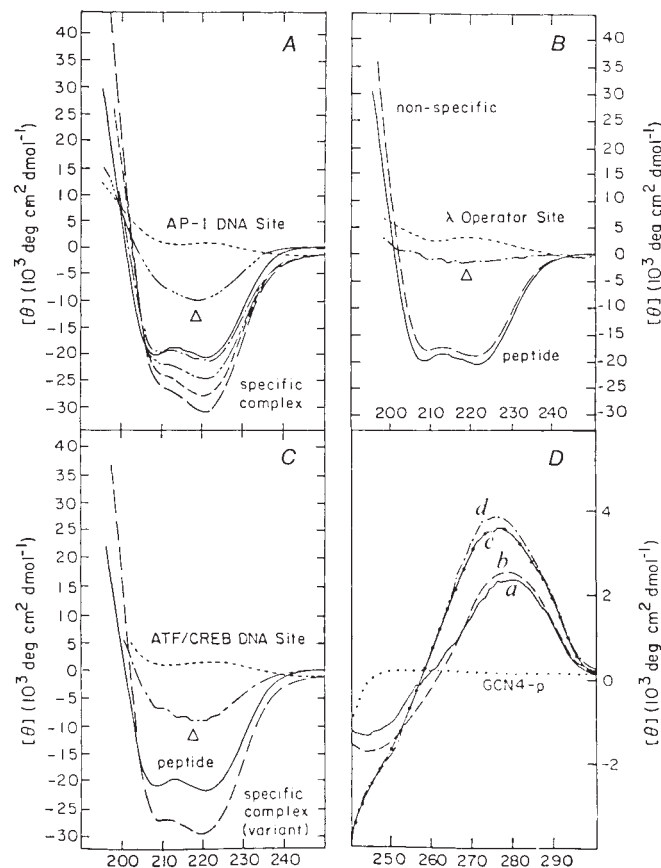


FIG. 3 A, Formation of the specific complex with a synthetic AP-1 site results in increased α -helix formation. The GCN4-p concentration was 34 μ M. Initial buffer conditions were 50 mM KCl and 12.5 mM potassium phosphate (pH 7.0) at 25 °C. —, the spectrum of GCN4-p is shown; ---, spectrum of the AP-1 site; —·—, spectrum of a 2:1 molar mixture of GCN4-p (one dimer per oligonucleotide duplex) and the AP-1 site (17 μ M); —·—·—, the calculated difference spectrum. Δ is calculated as the difference between the observed spectrum of the complex and the sum of the spectra of its constituents. The lineshape and magnitude of Δ is in accord with a coil-to-helix transition³⁰. Dissociation of the specific complex occurs at higher concentrations of KCl: spectra are shown at 500 mM KCl (—), 700 mM KCl (---) and 1,500 mM KCl (—·—). The 21-base-pair AP-1 site has sequence GAGATGAGTCATCTC; a similar DNA-dependent transition is observed with a consensus 15-base-pair AP-1 site. B, An unrelated control DNA site (the λ operator site *O*_L1) does not appreciably affect the helix content of GCN4-p. —, the spectrum of GCN4-p; ---, the spectrum of the λ operator; —·—, the spectrum of a 2:1 molar mixture of GCN4-p and the λ site; —·—·—, the calculated difference spectrum (defined in A). The sequence of the λ operator site is GATACCACTGCGGTGATATC and its complement. C, The ATF/CREB DNA site, which contains an additional GC base pair in the centre of the recognition site, induces a similar increase in helix content on formation of a specific complex. —, the spectrum of GCN4-p; ---, the spectrum of the ATF/CREB site (GAGATGACGTCATCTC) additional central base-pair underlined; the —·—, the spectrum of a 2:1 molar mixture of GCN4-p (one dimer per oligonucleotide duplex) and the ATF/CREB site —·—·—, the calculated difference spectrum (Δ). D, Conformation of the AP-1 (spectrum a; 15-base-pair sequence described in A) and ATF/CREB (spectrum b; related 16-base-pair sequence shown in C) oligonucleotides may be observed in the near ultraviolet region of the CD spectrum. The ellipticity of GCN4-p in this region (dotted line) is near zero. The spectrum of the AP-1 complex (c) and ATF/CREB complex (d) show similar small changes in amplitude and position of the local maximum.

DNA helix and forms contacts that, in part, determine the operator specificity of the repressor²³. In GCN4, the entire recognition region seems to undergo a folding transition on binding DNA. This can be a rather general mechanism for reducing the kinetic barriers in the assembly of protein-nucleic acid complexes.

Our results do not specify the details of α -helical packing in the basic region of GCN4. In the scissors grip model proposed for this class of DNA-binding proteins²⁰, the basic region forms an α -helical extension of the leucine-zipper, with a kink near the N-terminus. Although consistent with such a model structure, our data are also consistent with an array of α -helical segments within the basic region. Our data do, however, show that the basic region of GCN4 is almost completely α -helical when bound to DNA and that any kinks or turns in the peptide backbone involve very few residues. □

Received 30 May; accepted 15 August 1990.

1. Pabo, C. O. & Sauer, R. T. *A. Rev. Biochem.* **53**, 293–321 (1984).
2. Klug, A. & Rhodes, D. *Trends Biochem. Sci.* **12**, 464–467 (1987).
3. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. *Science* **243**, 1681–1686 (1989).
4. Hope, I. A. & Struhl, K. *Cell* **43**, 177–188 (1985).
5. Hope, I. A. & Struhl, K. *EMBO J.* **6**, 2781–2784 (1987).
6. O'Shea, E. K., Rutkowski, R. & Kim, P. S. *Science* **243**, 538–542 (1989).
7. Hope, I. A. & Struhl, K. *Cell* **46**, 885–894 (1986).
8. Oas, T. G., McIntosh, L. P., O'Shea, E. K., Dahlquist, F. W. & Kim, P. S. *Biochemistry* **29**, 2891–2894 (1990).

9. Kouzarides, T. & Ziff, E. *Nature* **340**, 568–571 (1989).
10. Sellars, J. W. & Struhl, K. *Nature* **341**, 74–76 (1989).
11. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. *Science* **246**, 922–926 (1988).
12. Agre, P., Johnson, P. F. & McKnight, S. L. *Science* **246**, 922–926 (1989).
13. Weiss, M. A. *Biochemistry* (in the press).
14. Oliphant, A. R., Brandl, C. J. & Struhl, K. *Molec. cell. Biol.* **9**, 2944–2949 (1989).
15. Hai, T., Liu, F., Allegretto, E. A., Karin, M., & Green, M. R. *Genes Dev.* **2**, 1216–1226 (1988).
16. Sadler, J. R., Sasmor, H. & Betz, J. L. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6785–6789 (1983).
17. Arndt, K., Boschelli, F., Lu, P. & Miller, J. H. *Biochemistry* **20**, 6109–6118 (1981).
18. Buck, F., Ruterjans, H. & Beyreuther, K. *FEBS Lett.* **96**, 335–338 (1978).
19. Wade-Jardetzky, N. et al. *J. molec. Biol.* **128**, 259–264 (1979).
20. Vinson, C. R., Sigler, P. B. & McKnight, S. L. *Science* **246**, 911–916 (1989).
21. Gartenberg, M. R., Ampe, C., Steitz, T. A. & Crothers, D. M. *Proc. natn. Acad. Sci. U.S.A.* **87**, 6034–6038 (1990).
22. Oakly, M. G. & Dervan, P. B. *Science* **248**, 847–850 (1990).
23. Jordan, S. & Pabo, C. O. *Science* **242**, 895–899 (1988).
24. Rosenberg, A. H. et al. *Gene* **56**, 125–135 (1987).
25. Studier, F. W. & Moffat, B. A. *J. molec. Biol.* **189**, 113–130 (1986).
26. Bowie, J. U. & Sauer, R. T. *Biochemistry* **28**, 7139–7143 (1989).
27. O'Shea, E. K., Rutkowski, R., Stafford, W. F. III & Kim, P. S. *Science* **243**, 1689–1694 (1989).
28. Lehrer, S. S., Qian, Y. & Hvidt, S. *Science* **246**, 926–928 (1989).
29. Chen, Y.-H., Yang, J. T. & Chou, K. H. *Biochemistry* **13**, 3350–3356 (1974).
30. Johnson, W. C. Jr. *Protein Secondary Structure and Circular Dichroism: a Practical Guide* 205–214 (1990).

ACKNOWLEDGEMENTS. We thank S. Lehrer (Boston Biomedical Research Institute) for discussion and use of his CD spectropolarimeter; H.T. Keutmann and S. Magil (Massachusetts General Hospital) for peptide characterization; K.A. Mason for technical assistance; and J. Habener, H. Kronenberg, and members of our laboratories for helpful discussion. This work was supported by the NIH (M.A.W., S.C.H. and K.S.) and the Markey Charitable Trust at Harvard Medical School and by the Howard Hughes Medical Institute. M.A.W. is supported in part by the Pfizer Scholars Program for New Faculty and the American Cancer Society. T.E.E. is supported by a postdoctoral fellowship from the NIH. C.R.W. is supported by a postdoctoral fellowship from Merck, Sharp & Dohme. The NMR spectrometer at Harvard Medical School is supported by NIH shared instrumentation grant 516RR04862-0 and the National Health Resources Foundation.

Phylogenetic and genetic evidence for base-triples in the catalytic domain of group I introns

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UNDERSTANDING the mechanisms by which ribozymes catalyse chemical reactions requires a detailed knowledge of their structure. The secondary structure of the group I introns has been confirmed by comparison of over 70 published sequences^{1–4}, by chemical protection studies⁵, and by genetic experiments involving compensatory mutations^{2,6,7}. Phylogenetic data can also be used to identify tertiary interactions in RNA molecules. This was first done by Levitt⁸, who predicted tertiary interactions in transfer RNA, which were subsequently confirmed by X-ray crystallography⁹. More recently, sequence comparison data have been used to predict tertiary interactions in ribosomal RNA¹⁰. We have searched a complete alignment of the core regions of group I introns^{1,2} for evolutionary covariations that could not be ascribed to classical Watson-Crick or wobble base pairings. Here we describe two examples of phylogenetic covariation that are most simply explained by postulating hydrogen-bonded base-triples similar to those found in tRNA. Genetic experiments with the *Tetrahymena* and *sunY* introns confirm the importance of these interactions for the structure of the ribozyme.

Comparison of group I intron sequences reveals a striking covariation of the second and third base pairs of stem P4 and the first and second nucleotides, respectively, of the single-stranded segment J6/7 (see Fig. 1a). Base pair two of stem P4 is most often G·C or C·G; when P4-2 is G·C, the first base in J6/7 is always U; when P4-2 is C·G, J6/7-1 is usually G, less often C, but never U. Several independent examples of concerted changes at these positions are seen in phylogeny (Fig. 1b), indicating a functional requirement as opposed to mere

evolutionary drift. For example, G·C-U changes to C·G-G within both distinct subclasses of the group I introns, showing that this triple mutation has occurred at least twice during their divergence. Similarly, P4 base pair 3 is usually C·G or G·C, and these base pairs are associated with C or U, respectively, at position J6/7-2; again, independent concerted changes of these positions are seen in phylogeny.

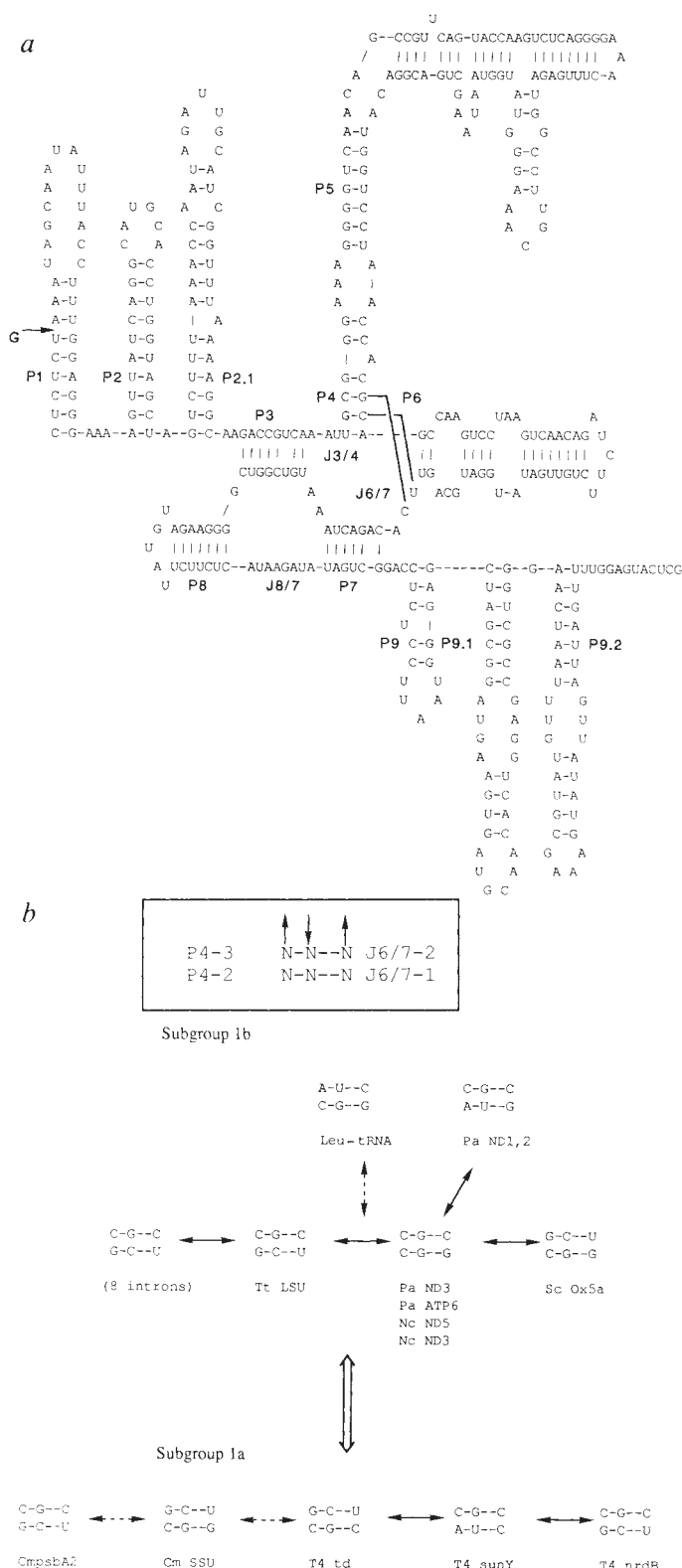
Comparing closely related introns can aid in distinguishing between sequence changes that are due to functional necessity as opposed to evolutionary distance. Two bacteriophage T4 introns, T4 *td* and T4 *sunY*, are virtually identical within the conserved core of group I introns¹¹, yet differ at the second and third base pairs of P4 and the second nucleotide of J6/7. It is unlikely that these sequences would have covaried if the base substitutions were merely the product of neutral drift.

To assess the chemical feasibility of the interactions detected by covariation, we have examined physical models of the proposed triples. All of the phylogenetically related base triples can be modelled as geometrically equivalent, or near-equivalent, structures (Fig. 2). In most cases, hydrogen bond donors and acceptors are interchanged without affecting the geometry of the interaction. The base-triples we propose between P4 and J6/7 are similar to those seen in tRNA^{8,9}, in that a single base interacts with one base of a Watson-Crick base pair on the major groove face of the base pair, with all three bases being roughly coplanar.

The small number of base changes between the bacteriophage T4 introns *td* and *sunY* made these introns ideal for initial tests of our model. If P4 and J6/7 do interact in a functionally significant manner, changes in one or the other of these regions should be deleterious, whereas changing both together should restore the original structure and enzymatic activity. We started with *sunY* and constructed all possible intermediates on the way to a *td*-like ribozyme, varying J6/7 and the base pairs of P4. The splicing activity of these intermediate forms was measured in the presence of saturating guanosine and the initial velocity of the reaction was determined (Fig. 3). Both intermediates that replace one base triple from *sunY* with the *td*-like base triple are slightly more active than the *sunY* wild type. In contrast, all mutants in which a predicted base-triple is disrupted have decreased activity. Loss of a hydrogen bond from a predicted base-triple (for example, C·G-C to C·G-U) reduces activity

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by 2- to 5-fold, whereas introducing a steric clash (for example, C·G-C to G·C-C) reduces activity by 9- to 17-fold.

These results also suggest that enzymatic activity is dependent on cooperative interactions between the two levels of triples. The constructs in which only one of the two *sunY* base triples becomes *td*-like, splice 1.3 and 2.8 times faster than *sunY* itself, respectively, whereas the fully *td*-like construct, in which both base triples have been altered, splices almost 14 times faster than the wild-type *sunY* control.

To test further the validity of our structural model, we constructed a variety of base substitutions in a truncated variant of the *Tetrahymena* ribozyme, pAG100, which is particularly sensitive to structural perturbations¹². As part of a previous study of phylogenetically conserved positions in the catalytic core, we had used cassette mutagenesis to generate all possible single mutations in P4 and J6/7, and all possible double mutations in P4 (ref. 2). Single mutations that disrupt base pairs in P4 are extremely deleterious. Compensatory mutations designed to restore Watson-Crick base pairing sometimes partially restore activity, but are much less active than the wild type. These experiments show that base pairing in P4 is necessary but not sufficient for full enzymatic activity, and support the idea that these base pairs are involved in important tertiary interactions (similar results have recently been obtained by Flor *et al.*, ref. 7). The few allowed alternate base pairs in P4 reproduce configurations already found in phylogeny: for example, changing P4-3 from C·G to A·U, and thus changing the base-triple from C·G-C to A·U-C, results in a configuration seen in the plant chloroplast tRNA leucine introns. Structurally, this substitution switches the hydrogen bond acceptor for the C4 amine of cytosine from the O6 of guanosine to the O4 of uracil.

We used our library of single and double substitution mutations to construct multiple substitutions involving P4 and J6/7. Triple substitutions that would seem to be chemically feasible are generally no more active than their double- and single-substitution parents. To determine why most triple substitutions have only a fraction of wild-type activity, we examined the possibility that only certain pairs of base-triples are compatible, as suggested by our experiments with the *sunY* intron. We constructed a variety of multiple substitutions that simultaneously changed both P4 base-triples (Table 1) by combining double and quadruple mutations in the P4 stem with single or double substitutions in J6/7. In contrast to substitutions that affected only one base-triple, striking suppression of parental phenotypes was seen when both base-triples were altered. The hextuple mutant G·C-U/C·G-G (*ScOx5a*-like) is particularly impressive, as substitutions that had little or no activity in isolation were restored to nearly wild-type levels when combined. No combination of bases on the way to this set is as active as the hextuple mutant itself. Several other suppressions where the activity of a hextuple substitution was greater than either triple substitution were also found.

TABLE 1 Activities of substitutions in P4-2·J6/7-1 and P4-3·J6/7-2

Upper level P4-3·J6/7-2	Lower level P4-2·J6/7-1			
	G·C-U	C·G-U	C·G-C	C·G-G
C·G-C	100 (Tth)	1.2	2.3	1.7
A·U-C	5.6	8.5	16	18 (Leu-tRNA)
G·C-U	0.5	3.6	18 (td)	90 (ScOx5a)
G·C-G	0.1	6.0	—	—

Mutants were constructed in a variant of the Tt LSU intron, pAG100 (described in ref. 12). Assay conditions are as in ref. 2, except that 5 mM spermidine was included to enhance the efficiency of the reaction. Values are expressed as a per cent of the wild type activity. Phylogenetic variants corresponding to the substitutions introduced at these positions are listed in parentheses. A dash indicates value not determined.

FIG. 1 **a**, The sequence of the catalytic domain of the *Tetrahymena* ribozyme displayed in the universally conserved secondary structure of the group I introns. Paired stems Pn and joining segments Jn/n are labelled according to ref. 13. The proposed base-triples are indicated by bold lines. **b**, Phylogenetic evidence for tertiary interactions within the catalytic domain of the group I introns. The P4 and J6/7 components are drawn to illustrate the proposed base-triples. Introns are arranged in an unrooted phylogenetic tree (F. M., manuscript in preparation), with full arrows indicating a close relationship and dashed arrows a more distant and hypothetical one. Independent instances of triple mutations that change all bases of each of the proposed base-triples can be seen. Names of introns and sequence data are from ref. 1.

The results from *Tetrahymena* and *sunY* lead to several conclusions. First, the structural integrity of a given base-triple is important for catalytic activity. Each of the genetically equivalent triples can be modelled as a chemically feasible structure with rough geometric equivalence (Fig. 2). Combinations of bases that are predicted to lack hydrogen bonds or contain steric clashes are less active than those containing at least one hydrogen bond. Second, it is even more apparent in the *Tetrahymena* intron than in *sunY* that there are cooperative interactions between the base-triples. In all the examples of strong genetic suppression, both triples were coordinately changed. If the effects of mutants in different triples were independent, these recombinant ribozymes should have had even less activity than their parents. Finally, we note that the various successful combinations almost all have corresponding representations in phylogeny (compare with Fig. 1b), whereas the less active combinations are not found in nature.

To determine whether the base-triples we have identified are important for all the steps in the splicing cascade, we also constructed a series of mutations in pSZ241 (ref. 12), which contains the wild-type *Tetrahymena* intron flanked by wild-type exon sequences. The substitutions introduced in pSZ241 were identical to several of the hexuple mutants constructed in pAG100 on the basis of evolutionary guidance (Fig. 1b). Several of the quintuple or hexuple substitutions (for example, the *td*-like and *ScOx5a*-like combinations) are more active than the quadruple, double and single mutations that went into their construction (data not shown).

The genetic evidence for strong interactions between adjacent base-triples is most simply interpreted as being due to stacking interactions between parallel, stacked base-triples. Such stacked base-triples can readily be modelled as two consecutive elements of a triple helical stack consisting of a classical double-stranded helix and a third strand with approximate A-form geometry interacting in its major groove.

The tertiary interactions that we propose are supported by three lines of evidence. Inspection of a phylogenetic database revealed covariation between the identities of certain base pairs and bases that have fixed locations with respect to the invariant secondary structure. The quality of phylogenetic data is subject to degradation through the inclusion of catalytically inactive introns, and because of sequencing and alignment errors; in

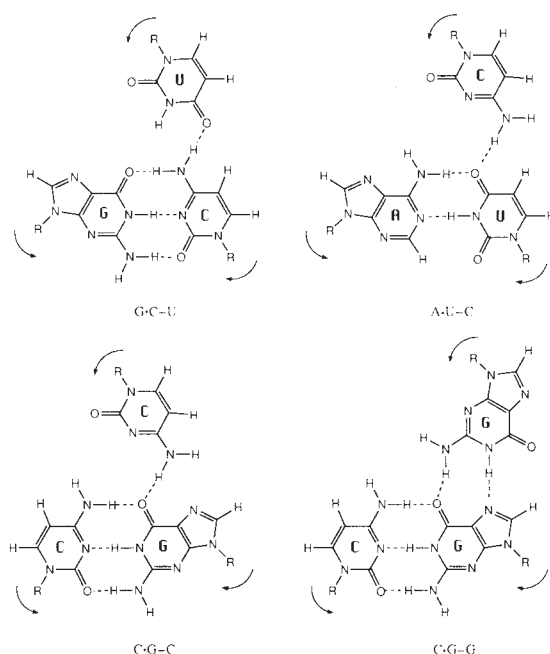


FIG. 2 Models of postulated base-triples discussed in text. Arrows indicate the polarity of the phosphodiester backbone.

P4 J6/7			
C·G-C A·U-C A·U	C·G-U A·U-C A·U	G·C-C A·U-C A·U	G·C-U A·U-C A·U
1 (SY41) <i>sunY</i>	0.64 (SY45)	0.16 (SY46)	1.35 (SY53)
changes in P4-3/J6/7-2			
C·G-C C·G-C A·U	C·G-U C·G-C A·U	G·C-C C·G-C A·U	G·C-U C·G-C A·U
2.85 (SY47)	0.61 (SY51)	0.83 (SY48)	14.3 (SY50) <i>td</i> -like
changes in P4-2/J6/7-1			

FIG. 3 Mutations in the P4 and J6/7 components of intron *sunY* and their effects on rates of 5' cleavage. The P4 stem (left) and first two nucleotides of the J6/7 segment (right) are shown for RNAs SY41 (*sunY* wild type) to SY50 (identical to the *td* intron over P4 and J6/7).

METHODS. Mutations were constructed²⁴ in plasmid SY41 (identical to mutant 4 in Table 1 of ref. 15), which contains the entire core of the *sunY* intron (structural elements P1-P9), and flanking exon sequences, cloned downstream of a T7 RNA polymerase promoter. T7 transcripts of *Dra*-digested plasmid DNA were purified on 4% acrylamide-8 M urea gels. Transcripts were preincubated for 20 min at 45 °C in 50 mM Tris-HCl pH 7.5 (at 20 °C), 50 mM NH₄Cl, 7.5 mM MgCl₂, 0.02% SDS. Reactions were started by addition of GTP to a final concentration of 2 mM. Samples were analysed on 5% acrylamide-8 M urea gels. The radioactivity in electrophoretic bands was measured directly with a Betagen scanner (Waltham, Massachusetts) and converted to molar amounts by correcting for the number of G residues. Numbers are initial rates of cleavage at the 5' intron-exon junction, normalized to wild type, calculated from the time needed to convert 25% of precursor molecules to products (~20 min for wild-type transcripts; all reactions were followed for 90 min, and 25% reaction times estimated either by interpolation or extrapolation, assuming linear kinetics between 0 and 25% reaction). Observed differences in initial rates were exaggerated at lower Mg²⁺ (6 mM), and suppressed at higher Mg²⁺ concentration (30 mM). All mutants yielded the same pattern of splicing products.

addition, not all tertiary interactions need be universally conserved. In view of these potential problems, the correlations that we have observed are particularly striking. Second, we found that these correlated sets of nucleotides could be represented as evolutionary transitions between roughly isosteric base-triples. It is important to note that the phylogenetic data is purely a statistical correlation, independent of any structural consideration, so that the finding that these data fit a consistent structural interpretation constitutes independent evidence in favour of the proposed interaction. Finally, we have used extensive genetic suppression analysis to confirm the importance of these interactions in two quite different group I introns, the group Ia intron *sunY* and the group Ib intron from *Tetrahymena*. Although we believe that these interactions are most simply explained by the formation of base-triples, it is not possible to exclude indirect interactions mediated by water molecules, metal ions or intervening nucleotides. □

Received 24 April; accepted 2 August 1990.

- Cech, T. R. *Gene* **73**, 259-271 (1988).
- Couture, S. *et al. J. molec. Biol.* in the press.
- Michel, F., Jacquier, A. & Dujon, B. *Biochimie* **64**, 867-881 (1982).
- Davies, R. W., Waring, R. B., Ray, J. A., Brown, T. A. & Sczacchio, C. *Nature* **300**, 719-724 (1982).
- Inoue, T. & Cech, T. R. *Proc. natn. Acad. Sci. U.S.A.* **82**, 648-652 (1985).
- Williamson, C. L., Tierney, W. M., Kerker, J. & Burke, J. M. *J. biol. Chem.* **262**, 14672-14682 (1987).
- Flor, P. J., Flanagan, J. B. & Cech, T. R. *EMBO J.* **8**, 3391-3399 (1989).
- Levitt, M. *Nature* **224**, 759-763 (1969).
- Klug, A., Ladner, J. & Robertus, J. D. *J. molec. Biol.* **89**, 511-516 (1974).
- Woese, C. R. & Gutell, R. R. *Proc. natn. Acad. Sci. U.S.A.* **87**, 663-667 (1990).
- Shub, D. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 1151-1155 (1988).
- Doudna, J. A., Gerber, A. S., Cherry, J. M. & Szostak, J. W. in *Cold Spring Harbor Symp. quant. Biol.* **52**, 173-180 (1987).
- Burke, J. M. *et al. Nucleic Acids Res.* **15**, 7217-7221 (1987).
- Kunkel, T. A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 488-492 (1985).
- Michel, F., Netter, P., Xu, M.-Q. & Shub, D. *Genes Dev.* **4**, 777-788 (1990).

ACKNOWLEDGEMENTS. We thank J. A. Doudna and R. Green for oligonucleotides, P. Dumas, E. Westhof and J. M. Cherry for help with molecular modelling. This work was supported by Hoechst AG.

Communication problems

Michael Buckingham

The gap between the promise of electronic information systems and practice is shrinking. But slowly.

FOR almost two decades we have been threatened with a revolution in the communication of technical information to be brought about by electronics. So far, however, the casualties have been confined to the revolutionary armies themselves.

One notable example is the American Medical Association, which is pulling out of a scheme to run an on-line information service for its members. With losses reportedly running at \$250,000 a month, nobody can accuse the AMA of not trying. The venture did not fail from lack of initial interest; registrations were well up to expectations. Rather, few potential users were prepared to overcome the 'hassle factor' of telecommunications with any regularity. The point is this: if an organization with the money, power and influence of the AMA, and an audience well able to claim tax relief on expenditure, cannot make such a service work, what hope is there for penetration of electronic systems into the beleaguered academic market?

The importance of convenience is highlighted by the interest in professional services reported by the French Minitel operation, which offers a freely distributed base of five million Minitel videotex terminals. Access to the Medline database in the United States through Minitel is now being heavily promoted, with telephone companies in Germany, Italy and The Netherlands planning to join in the distribution of terminals free or at low cost. (By contrast, the value of supporting an information and communications infrastructure has so far been lost on the British government.)

Research results

Developments such as these have had little bearing on the main area of concern for the scientist, the communication of primary research results. The principal arguments for electronic systems — rapid communication and rapid response — still hold good, and are strongest when information can be presented concisely and where the audience has the courage to respond off the cuff. But there remains a mismatch between the volumes of information considered essential in a scientific paper and the volumes which can easily be handled by computer networks and conveniently printed. The merits of short communications must be clear to readers of this journal at least — if not its contributors.

This is a challenge to which technology may be rising faster than expected. The arrival of high-speed digital communications in the form of integrated services digital networks (ISDNs) holds the possibility of transmission of papers in image form at high speed and acceptable cost. One consequence may be that the much-discussed Adonis project (see *Nature* **344**, 287; 1990), which is based on the delivery of papers in image form using the medium of optical disks, will be outdated even before it is fully commercialized.

High-speed digital communications will certainly benefit full-text databases, which so far have grown steadily but not spectacularly. The US database hosts BRS and STN have continued to put large resources behind building collections in medicine and chemistry; BRS, for example, now has over 90 titles in its collection. This number is small in comparison to the 400 titles planned for Adonis which, in turn, is only a fraction of the total biomedical literature. Yet the volume of material that Adonis can carry is still constrained by the physical problems of journal scanning, disk mastering and distribution.

A considerable step forward in providing a broader service would be to integrate the efforts that now go into the collection and processing of journal issues for major abstracting services with the scanning and distribution on demand of papers in image form through ISDNs. This would both increase the volume of titles and free the distribution process from the need for physical access to an Adonis workstation. Although Adonis has now moved to industry-standard hardware, the physical constraints remain substantial.

The main question to be considered is a political one: who would control an integrated service such as this? Capital and system development would be substantial and it is not at all clear that it would be possible to support many competing systems. The real importance of Adonis may be that it has served to unite a collection of publishers into a group able to counter the power of on-line hosts. Whether a hard-pressed library community will be able to combine to represent the consumer remains to be seen.

What of the 'end user', the scientist at the bench? The costs and complexities of access to on-line services have combined to hold back growth in this market, the information professional continuing to be

the gatekeeper. Disk-based services have, however, grown rapidly; for example the penetration of Medline CD-ROMs into hospitals and medical research centres is now close to complete, although researchers in the basic sciences are generally less favoured.

Modern methods of data retrieval are undoubtedly more comfortable to use than manually thumbing through printed indexes, but the indexing and retrieval process has not advanced to any comparable degree. The sheer volume of material remains overwhelming. Like motorists we are locked into a situation with better and better cars travelling more and more slowly on more and more crowded roads. Against this background, the growth in various forms of print-on-paper review publications is hardly surprising — the immediate need is not for more information, but for selection, evaluation and analysis.

Social networks

At the heart of the matter is a failure to build effectively on existing foundations. Scientific meetings are said to be where you go to meet people you don't know you need to meet. An electronic service will represent a real advance when it can help you find out what you don't know you need to know. To do so it must combine exchange of formal knowledge with the cut and thrust of debate. In other words, electronic networks need to be built on social networks.

As in so many other fields, the model approach could well be found in Japan. The National Academic Centre for Science Information Systems (NACSIS) in Tokyo provides a private network linking universities, colleges and national research institutes. Besides providing basic bibliographic services and access to conventional databases, NACSIS offers facilities for the exchange of research work by electronic mail, bulletin boards and fax, and in its breadth of coverage and attention to customer needs is now going well beyond the similar types of networks to be found elsewhere. The combination of convenient access to the formal literature and informal communication within a relatively tight-knit community will prove enormously powerful.

But the nature of the communication process in science, which still rests upon peer review and the delivery of the printed word, remains largely unchanged. Considering the interval between the invention of printing and the advent of the first scientific journal, we have all been over-optimistic in welcoming the New Age of information. □

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Choice for the consumer

Peter Sheterline

Current Opinions in Cell Biology. Editors N. B. Gilula and L. Wolpert. *Current Science*. 6/yr. North America \$185 (institutional), \$85 (personal); Japan ¥41,250 (institutional), ¥18,750 (personal); elsewhere £110 (institutional), £50 (personal).

THERE are two extreme ends to the spectrum of philosophies regarding the publication of scientific data (and of anything else for that matter): the Calvinist view which regards publication of anything less than a 'complete' piece of work as opportunistic; and the liberal view that all (refereed) experimental information has value and that any other position smacks of censorship. For publishers, of course (excluding those mediating the activities of scientific societies), the latter position is implicit in the process of running a publishing business, providing that the publications can be sold; for administrators of libraries, quite the opposite view is convenient in the context of diminishing resources (see Merrimen's article in last year's journals issue, *Nature* **341**, 349–350; 1989). For the scientific community itself, and therefore for the benefits to society that accrue from science, the forces that determine 'quality' and quantity of publication are complex, as in the ecology of science, the number of papers published, the ability to obtain funds to do the research leading to publication and the career opportunities for individual scientists are all interlinked. Paradoxically, the suppliers of information in this publishing system are also the consumers and the

objectives in each role may be different. Thus, although like all ecologies it is intrinsically self-adjusting and basically conservative, the increasing pressure for individuals to publish may have driven the system to one that benefits the supplier but not necessarily the consumer.

The size of the professional scientific community, and hence of its output of data, has grown out of all recognition from the community of gentleman (mostly) scientists of the nineteenth century. This, and the increasing pressure on scientists to publish regularly to avoid perishing, has led to the large increase in the number of scientific journals (as evidenced by this annual section in *Nature*) and a steady shift away (in terms of numbers) from general to audience-specific journals. As has often been noted, this results in real difficulties for current awareness of scientific information and tends to drive the formation of self-appointed elites that influence the content of the 'high profile' journals and thereby determine fashionable paradigms. The counter force to these events has been the growing availability of computer-based search facilities and the emergence over the past few years of opinion and trends type journals. This, it seems, permits a victory for the liberals.

Cell biology, that amorphous discipline which has clasped morphology, biochemistry, biophysics and latterly molecular genetics to its bosom, illustrates well the evolution of proliferative publishing. The earlier trend towards specific journals on membranes, supramolecular structures(?), calcium and so on, while recognizing the concentration of effort in particular fields, did not easily satisfy the need for cell biologists to maintain some kind of holistic perspective on cell behaviour and function, arguably, the *raison d'être* for cell biology. Does the recent introduction of *Current Opinion in Cell Biology* and will the projected *Trends in Cell Biology* help redress the balance?

Many cell biologists will have already reached their own conclusions. The first issue of *Current Opinion in Cell Biology* was published at the beginning of 1989 and is modestly priced to encourage personal subscriptions. It was the brainchild of Vitek Trecz, a publishing entrepreneur, and emerged from a similar set of overview journals aimed at the medical community. The journal is released in six issues per annum, each of which contains current overviews of different areas of cell biology; for example, the first is entitled "Cytoplasm and cell motility", the second "Cell multiplication and cell regulation". Each subject area has a section editor who gathers together a group of relevant authors whose remit appears to be to comment on the current status in their discipline in the light of data published in the previous year. The authors are supported by a bibliography of "world literature" in the field from the previous year (in fact in the period of about 4–16 months before publication) gathered by an independent group of literature sleuths. The main bibliography, though large, is not complete, but has been sifted for relevance.

As a consumer scientist and teacher, I find this journal extremely useful. It is a rolling record of overview and comprehensive literature source, which can be conveniently picked off the shelf for entry into the literature base in any of the main areas of cell biology. I have enough shelf space for the next few years' worth. It is particularly useful for final-year undergraduates and for graduate students. I am less impressed by the overviews themselves, mostly because (presumably in deference to the journal's title) they are opinions. Although they are constrained in length (four printed pages) it would be more useful if they were used to establish the key information in the field as of then (perhaps we could call them reviews?) and to leave detailed discussion of particular aspects of the field (mainly of value to a relatively small band of specialists) to the excellent literature source. There would then be a rolling record of the current positions in the key areas of cell biology in

New journals review 1990

THE following explanation is offered to those wishing to know the basis on which the journals mentioned in the following pages were chosen for review.

In *Nature's* previous journal supplements*, two principles determined the time criteria for a publication to be eligible for review in a particular year. First, that at least four different issues should have been published, to give reviewers a reasonable sample on which to base a judgement. Second, that most new journals start as quarterlies though they often later become more frequent.

Thus in last year's review supplement journals first appearing after May 1987 and with four issues available by May 1989 were considered for review. The first date was carried forward from the previous year; the second meant that four issues of a quarterly would be available to reviewers at the time of commissioning reviews in June 1989.

Other criteria were that the journal should appear at least three times a year, and that the main language used should be English. Abstracts publications and news-

letters are not reviewed. Several titles eligible for review are not covered here; a list of them appears on page 599.

The brief given to the reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1990 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1990. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. □

* See *Nature* **341**, 350–370 (1989); **335**, 459–478 (1988); **329**, 357–376 (1987); **323**, 359–379 (1986); **317**, 293–308 (1985); **311**, 309–330 (1984); **305**, 477–497 (1983); **299**, 491–514 (1982) and **293**, 341–369 (1981).

one place and upon which new developments could be hung. Most areas could expect an airing annually or at least every two years, if the journal remains at its present size. Such review material, although reiteration of basic information to practitioners in the field, requires their wisdom for compilation. Such a potted literature service is, and will increasingly become, a useful companion for cell biologists. □

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Laboratory practices

Steve S. Sommer

Technique. A Journal of Methods in Cell and Molecular Biology. Editors Peter Little (UK) and Stephen Hughes (US). Saunders Scientific. 6/yr. US \$210 (institutional), \$125 (personal), \$85 (student); European Community £157 (institutional), £100 (personal).

Methods in Molecular and Cellular Biology. Editor in chief Bernard Perbal. Wiley-Liss. 6/yr. US \$125; elsewhere \$175.

HAVE methods become our madness? In the beginning of my scientific life, there was a strong oral tradition, a few established books and journals such as *Methods in Enzymology* and *Analytical Biochemistry*, and methods papers appeared only sporadically in the literature. Curiously, these papers were viewed by some as second-class publications. As the number of individuals engaged in molecular biology grew and the pace of research accelerated, a thirst for additional sources of methods developed. In 1982, Maniatis, Fritsch and Sambrook codified much of the previous oral tradition in their now classic "blue bible". A myriad of non-traditional sources appeared such as the "for the record" section in *Nucleic Acids Research*, and periodically updated protocols were published (such as Wiley's *Protocols in Molecular Biology*). Biotechnology suppliers such as BRL began publishing newsletters focusing on methodology, which were mailed free to anyone wanting a copy. They contained useful articles and they were a hit! Now, virtually all suppliers provide free newsletters, and journals such as *BioTechniques* have emulated this approach.

Has all this activity quenched our thirst for methods? The editors of *Technique* and *Methods in Molecular and Cellular Biology* (MMCB) are betting that it has not. *Technique* is a new journal that

publishes methodology reviews, subject overviews, protocols (bench-ready protocols of previously described methods), original contributions and short communications. Dates of receipt and acceptance are not provided so that the rapidity of publication cannot be assessed.

As stated in the inaugural issue of *Technique*, the goal of this journal is to publish "detailed descriptions of methods, discussions of sources of failure, sources of error, or ease of application". These goals are generally met. Most laudably, the articles are concise, and the protocols are boxed and written to be "bench friendly". The co-editors have assembled a stellar editorial board including an international array of individuals who have made seminal contributions to the methodology of molecular biology. But this board needs to be used more effectively. Some of the "original contributions" are at best minor modifications of previous methods which should have been published in the "protocols" section with citation of the previous work. New journals such as *Technique* and MMCB can establish credibility only by carefully separating the original contributions from rehashes of previous work.

MMCB publishes reviews, original contributions, commentaries on protocols (describing difficulties with or improvements to published protocols, applications of published protocols to other fields or requests for information on obscure

points in procedures), and "nuts and bolts" ("letters from readers wishing to comment on the quality, usefulness, and applicability of methods published in the journal and of commercial products in molecular biology"). For commentaries and nuts and bolts, replies from the authors and companies are generally published in the same issue.

There were only a few examples of commentaries and nuts and bolts in the four issues I was sent for review, and these were unimpressive, but it seems premature to pass judgement on their ultimate usefulness. Again, rapidity of publication cannot be assessed because the listed journal dates predate the acceptance dates on the manuscripts. The editorial board is broadly based, with representation from countries such as mainland China and Czechoslovakia. Few articles dealt with areas of my expertise so it was harder for me to judge the effectiveness of editorial review.

Each issue of *Technique* or MMCB contains six to nine articles. Both journals can offer sustenance to those who still thirst for methods. Only time will tell how much thirst will remain after partaking of the established brands. □

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Bid for the top

Jeremy Hyams and David Latchman

The New Biologist. Editor in chief Arthur S. Levine. Saunders. 12/yr. US \$125 (personal), \$85 (student), \$225 (institutional); elsewhere \$160 (personal), \$120 (student), \$260 (institutional).

The New Biologist was launched in October 1989 with the statement that "virtually all of the biological sciences are converging on two central phenomena, namely, signal transduction and differential gene expression" and the promise to "deliver to you significant interdisciplinary papers from a broad readership". Almost one year on, it is interesting to see how far the editors have justified the former and how close they have come to achieving the latter.

The New Biologist publishes three types of papers: research reports, research reviews and meeting reviews. In addition, there are occasional essays under the heading "Theory and hypotheses", and an "Editorial forum" which has varied from reviews of the 1989 biological Nobel prizes to a rather indulgent homily on scientific creativity. It is the balance or, perhaps more accurately, the imbalance, between these various headings that is most striking. Clearly, any new journal lives or dies by the quality and quantity of its original papers. Meeting reviews can be interesting and informative, but they are no substitute for high-quality original research reports. *The New Biologist* has tried to

cope with the slow paper flow that any new journal experiences by appointing an editorial board of truly Cecil B. de Milleian proportions. Given this, it is disappointing that the reasonable numbers of research papers in the early issues have declined significantly, with more recent issues containing only four such papers and, hence, dominated by the other sections.

Although the editors of *The New Biologist* must therefore have some qualms about the quantity of its output through its first year, the quality is extremely high. This is matched by the production which lies, perhaps not entirely coincidentally, somewhere between *Cell* and the *EMBO Journal*. Quite where *The New Biologist* sees itself in the cell and molecular biology journals market is in fact quite interesting. On the one hand it has made a serious, and to a significant extent successful, bid for the sort of top-of-the-line research that might hitherto have gone to the journals mentioned above or even graced these hallowed pages. On the other hand, the review articles are occasionally written in a style that owes more to a Murdoch tabloid ("The pinheads and fuzzbrains go haywire") than a serious heavyweight. The editors should be encouraged to keep their heads and maintain the high standards they have set for research articles. If the quality can be maintained the quantity will surely follow. □

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Growing pains

M. J. O. Wakelam

Cell Growth & Differentiation. Edited by George F. Vande Woude et al. American Association for Cancer Research. 12/yr. US \$150 (institutional nonmembers), \$90 (individual nonmembers).

Growth Factors. Editor in chief Antony Burgess. Harwood. 4/yr. North America \$190 (corporate), \$130 (university), \$66 (individual).

Progress in Growth Factor Research. Executive editor John K. Heath. Pergamon. 4/yr. DM 285.

INVESTIGATION into the mechanisms involved in cell growth is one of the most heavily funded areas of modern biomedical research. Thus it is hardly surprising that over the past few years the number of journals covering this area has also appeared to proliferate. The three latest additions, *Cell Growth and Differentiation*, *Growth Factors* and *Progress in Growth Factor Research* hope to fill perceived gaps in the present journal

field. To this end each has assembled editorial boards consisting of well-respected, active researchers which, not surprisingly, overlap somewhat. Each journal is also to be praised for not instituting page charges, a serious obstacle to British scientists since the agencies supporting us do not provide publication costs.

One of the impressive aspects about research in the fields of growth-factor structure, function and action is the interactions that have taken place between people working on the different aspects, such as between those studying the molecular biology of growth-factor action and those investigating the signal-transduction pathways. This degree of communication could imply that specialist journals may have a role in increasing such interactions. But the initial impetus in each case was the result of different groups publishing in widely read general journals in the first place, which would tend to argue against such a role for specialist journals.

Cell Growth and Differentiation is described as the molecular biology publication of the American Association of Cancer Research. A large proportion of

the papers in the first six issues deals with oncogenes, though the editors hope to encourage papers on other aspects of growth and differentiation research — indeed, the composition of the editorial board should help in this. Some issues contain a "research capsule", which is designed to provide an update on new concepts and methodologies, and which I found both informative and relevant. Nevertheless, it is difficult to see this journal making a major impact: most of the papers it has attracted so far and those it appears to be targeting are generally found in existing specialist journals such as *Oncogene*, *Oncogene Research*, *Molecular Cell Biology* and *Journal of Cell Biology*. There does not really seem to be a need for yet another journal in this area.

Growth Factors hopes to carry papers covering all aspects of research into molecules which control the proliferation and differentiation of cells. It seems to have been successful in this aim so far, having published papers on the genes, synthesis, purification and function of growth factors. Each issue carries a wide cross-section of interest, and a commendable aspect is the profusion of colour illustrations, of particular importance for immunohistochemical studies. But despite the journal's plan to focus on growth factors, one is left with a feeling of a lack of specialization. Most of the recent articles have tended to be concerned with polypeptide growth factors such as fibroblast growth factors, insulin-like growth factors and transforming growth factors. Perhaps this could become the primary focus of *Growth Factors*, otherwise there is a deal of similarity with several cell biology journals, which makes one doubt if there is a niche for this journal to occupy.

In addition to journal proliferation, the multiplication in the number of journals either devoted to, or carrying, reviews has got to the stage where it seems that a large proportion of the research community must be writing, or have recently written, a review. Thus is there a need for *Progress in Growth Factor Research*? Surprisingly, perhaps, I think there is. Despite the efforts of journals such as *Growth Factors* and *Cell Growth and Differentiation*, it is not possible to keep up with the literature covering all aspects of growth-factor research. There is a need for a journal containing up-to-date, short reviews to help today's somewhat narrow researcher to be aware of new developments and concepts in the wider area of growth factors. The first four issues of this journal carry reviews of topics ranging from clinical trials to oncogene structure and function. The reviews are written by active workers in their fields and seem to be published soon after preparation — it is refreshing to read a review which contains

references to papers published in the same year. The articles do not contain pages of history but do focus on the key, up-to-date pieces of information, which is what is required of a good review journal. *Progress in Growth Factor Research* appears only quarterly, which should maintain the topicality of its articles. Provided the present standard is maintained, this should be a useful journal for those in the field. □

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Antipodean issues

John Galloway

Today's Life Science. Editor Jeremy Knibbs. Thomson Publications, Australia. 12/yr. Australia A\$56, rates elsewhere available on application.

FOR a moment the title fooled me, but the advertising gave it away. "Life" is not to be understood in the sense of "life on Earth" but rather as in "life and death". This is not *Trends in Biological Sciences*, it is a collection of reviews of molecular medicine for those Australians who make their living at it. The best test for a readership of any journal or magazine is its advertising. *Today's Life Science* carries a lot of high-tech and bio-tech adverts and this I take it defines its audience. Although I couldn't help wondering how big this audience might be, the advertisers clearly think it is big enough.

The format is fairly glossy and the formula is simple — half a dozen reviews in each issue categorized by medical discipline: biochemistry, haematology and microbiology. The articles taken *en masse* reveal how outmoded are medicine's traditional disciplines and how arbitrary their boundaries in the age of molecular biology. A further review covers methods and the final one, "Agenda", addresses a problem that adds some other dimension to the straight science — ethical, educational or economic. Most issues of the journal also carry a feature called "Viewpoint" which as far as I could gather overlaps with "Agenda" in its choice of subjects, but its tone seems more partisan. A number of pages are also given over to "Current Topics" — short pieces mainly on science but leavened with political or social observation.

This is a journal rather than a magazine; the material is largely, perhaps exclusively, written by working scientists rather than by the editorial staff. Inevitably, it has a parochial feel to it. It is written by Australians for Australians, and an emphasis on Australian research is one of the guide-

lines for authors. Some of these authors are Australian by adoption rather than by accident of birth: I was glad to see an article about heparin by Joan Dawes who not that long ago was helping run the Scottish National Blood Transfusion Services Headquarters laboratory in Edinburgh.

The first issue covered receptor control of haemostasis, tumour necrosis factor, the molecular approach to a cholera vaccine, the pyruvate dehydrogenase locus and automated DNA sequencing. Later issues are similar. The agenda item for the first issue was "biomedical education and research in crisis": poor salaries, a lack of

form a concert or open a holiday resort.

I can see — or think I can see — why Australian molecular medicine might need a journal like this. It is really a sort of round robin keeping what is presumably a rather scattered tribe of scientists in some sort of contact with each other. Australia has some excellent molecular medicine and it is worth keeping in touch about.

However, the tone is a bit insular, a quality very much at variance with science's internationalism and with the outgoing character of Australian scientists. Why not have a regular feature from someone in the scientific world outside Australia? It also needs some decent



The headquarters building of the Australian Academy of Science in Canberra. The body of the building is contained inside a huge copper-sheathed dome 47.5 metres in diameter, resting on arches set in a moat.

career structure and funding problems. I couldn't think for the moment where I'd heard that before. In fact, a strong sense of *déjà vu* accompanies the reading of many of the articles that have strayed from straight science. A government gift horse well deserving of a long hard look in the mouth, for example; good Australian ideas not reaching the market place; priority elbowing out patents; the poor image of science on television. It is all pretty familiar.

One article that managed to break away to some extent from the utilitarian or from engaging in recrimination about government was a piece about Australian involvement in the human genome project. Not content with pointing out the enormity of the task of sequencing the three thousand million base pairs of human DNA involved, the authors suggest that what we really want to sort out is the genome of the total biosphere, all 10^{14} base pairs, before it is too late. A nice touch was the piece looking forward to a visit to Australia by Jim Watson — it could have been Frank Sinatra coming to per-

correspondence columns — the journal comes out once a month so this would certainly be feasible. To do it justice, the first issue did remind the readership to get in touch, but later issues I saw provided no evidence that anyone was taking any notice beyond contributing the articles. Finally, it also seems to be taking itself rather too seriously. Wasn't it Bertrand Russell who said "One of the symptoms of an approaching nervous breakdown is the belief that one's work is terribly important"? I suppose this is true of the scientific community as well as of individuals. It is worth considering that one reason young people don't find science very appealing is that it does not come across as a barrel of laughs — Kylie Minogue and Jason Donovan playing the young Marie and Pierre Curie might be an interesting experiment in communication and one that this journal could perhaps suggest to Australian television. □

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Proliferating titles

P. H. Gallimore

Cancer Cells. Editor Paula Kiberstis. *Cold Spring Harbor Laboratory Press*. 12/yr. US \$180 (institutional), \$55 (personal).
Cancer Communications. Editor in chief Alan C. Sartorelli. *Pergamon*. 6/yr. US \$180 (institutional), \$30 (personal).
Genes, Chromosomes & Cancer. Editors in chief Felix Mitelman and Janet D. Rowley. *Wiley-Liss*. 6/yr. US \$90.

THESE are three very different journals: the first consists almost exclusively of monthly reviews, the second is an open forum for short papers covering any aspect of the cancer research field and the third encompasses the specific area of cancer genetics from cytogenetics to DNA.

Cancer Cells is intended to cover all aspects of new developments, from basic science to clinical research. As the journal includes "commentaries, opinions, hypotheses and reviews", sometimes the scope and format of these articles overlap. Clearly one of the editors' aims is to create a more formalized version of a journal club and only time will tell if this goal is attainable in print.

I like the general layout, the uncomplicated manner of the illustrations and short but concise presentations. Most of the specialized cancer journals also publish short reviews. By virtue of its multidisciplinary nature, however, the field is vast and rapidly expanding; this new venture has succeeded in several novel ways in making a niche for itself. Meeting and book reviews, together with an extensive meetings and symposia calendar, are useful additions. For those who like to have a refresher course on the origins of oncogene and proto-oncogene acronyms each issue has an "oncogene file" in which one or two genes are defined, characteristics briefly summarized and a few key references provided.

Cancer Communications is a journal which aims to publish short research articles rapidly. The diverse spectrum of articles published so far makes this publication attractive only to research institutes or medical school libraries. There are a large number of established journals which are in direct competition with *Cancer Communications* and I think that this one will survive only if members of its illustrious editorial advisory board attract better quality science or submit a proportion of their own data for publication here.

Genes, Chromosomes and Cancer is also in a very competitive area, the competition coming not only from traditional

cancer and genetics journals but from prestigious publications like *Cell*, *Science*, *Nature* and *Proceedings of the National Academy of Sciences*, to name but a few. Nevertheless, I think that this journal has got off to an excellent start by attracting good papers from most of the top laboratories in the field. The editors hoped that this would be a venue for rapid publications, but I note that some papers have taken 4–6 months following acceptance to get into print; this must be improved. The layout of this journal is neat but the quality of some of the illustrations showing banded chromosomes is barely acceptable. Some attention needs to be paid either to the primary submissions or to

the quality of the journal's paper.

The realization of the number and diversity of genes involved in cancer and the complex nature of the cytogenetic changes associated with progression of the disease ensures a fertile field for this publication, at least in the short term.

The title of this review reflects my growing anxiety and frustration that the expansion of new titles in this field makes the task of keeping up with the literature an increasing problem. □

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Mixture for the market-place

D. Swirsky

Leukemia and Lymphoma. Editor in chief Aaron Polliack. *Scientific and Technical Book Services*. 6 issues/volume. £34 (personal), Dfl.400 (library), Dfl.532 (company).

PROBING the molecular events which culminate in haematopoietic malignancies is quite properly the leading edge of research on leukaemia and lymphoma. Such work is distributed through various established quality journals, both scientific and medical. *Leukemia and Lymphoma* does not profess to compete for readers on those terms, but aims fairly and squarely at "providing a journal for those practising physicians and scientists involved with laboratory and clinical diagnosis, clinical care and management and therapy of patients with leukemia and lymphoplasma-cytic disorders". It is all too easy to forget that leukaemias and lymphomas are lethal diseases affecting 24 per 100,000 children and adults a year. Day-to-day decisions on the diagnosis and treatment of these conditions need to be as well informed and accurate as possible. We take it for granted that patients want the best available treatment. Physicians genuinely wish to provide it.

Leukemia and Lymphoma is setting out its stall in a very busy market-place. Competition in the field is ferocious — *Leukemia* and *Leukemia Research* are pitched directly at the same customers; *Blood*, *British Journal of Haematology*, the *European Journal of Haematology* and *Bone Marrow Transplantation* are general competitors. Add the cancer, cytogenetics and pathology journals, and the task of launching a new journal in this field falls into perspective. The problem will not be of too few papers to fill the journal (the "publish or perish" pressures will ensure a steady flow);

rather, it will be to compete for the best quality papers.

The editors are drawn from 10 countries, with only the United Kingdom boasting two representatives. Their strategy in the first six months has been to provide two to four high quality reviews per issue, allowing wide-interest subjects to be placed alongside more specialized topics. Autologous bone-marrow transplantation in acute lymphoblastic leukaemia, interferon- α in chronic granulocytic leukaemia and multidrug resistance rub shoulders with two refreshing contributions on leukaemia in tropical Africa. The editors have contributed substantially to the first volume, a glance through volume 1 of *Blood*, published in 1946, revealing a prestigious precedent.

The original articles are equally mixed: oncogenes and DNA genotyping, clinical trials, cytogenetics, electron microscopy and monoclonal antibodies all get space. Epidemiology is a welcome addition — a case-control study linking small family size and glue exposure to non-Hodgkins lymphoma, and agricultural chemicals to acute lymphoblastic leukaemia in the north of England.

Given current financial strictures, will this journal sell? I hope so. At the moment it has something for everyone, but perhaps its best bet will be to create a specific niche in the border area between haematology, clinical pathology and histopathology. Given the recent advances in immunochemical, cytochemical and molecular methodology to characterize haematopoietic cells in blood, tissues and cultures, and technical advances in light, confocal and electron microscopy, subject matter is not lacking. The reputation of the editor in chief places him in a strong position to attract good papers in this area, and the strength of the editorial board should ensure the journal's future. □

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Wide definition

K. R. Bruckdorfer

Journal of Lipid Mediators. Editor in chief B. B. Vargaftig. *Elsevier*. 6/yr. US \$210; elsewhere Dfl. 373.

AS THE boundaries between the classical scientific disciplines continue to erode and the number of publications increases, it is virtually inevitable that new specialist journals will appear, especially in areas identified as rapidly growing. It will be intriguing to see how the newcomers fare against the generalist journals in attracting the best-quality papers. The problem for the specialist journals is that contributors almost always study the particular specialism of the journal against another specialist background, and thus such contributors may sometimes have difficulty in deciding where to send their papers.

Journal of Lipid Mediators was launched in January 1989 and there are now about eight issues. The journal aims to carry articles on the chemistry, biophysics, biochemistry, pharmacology, toxicology, pathology, immunology and clinical aspects of lipid mediators. Lipid mediators are defined by the journal as lipid derivatives from all sources and include lipid-soluble vitamins, phospholipids, lipoproteins, lipopolysaccharides or fatty acids and their metabolites and derivatives. Synthetic lipids with biological activity are also considered. The definition seems to be surprisingly broad given the existence of some excellent journals of long standing that also cover the lipids-lipoproteins area, including at least one published by the same publisher as that of the new journal.

Analysis of the articles in the journal, however, shows a different picture. In the four volumes of this year, nine out of thirteen original articles concern platelet-activating factor. In another section, there are abstracts from the International Union of Pharmacology satellite conference on platelet-activating factor held in Paris in June of this year. The other articles are about diacylglycerols and phospholipase A₁, and there is a review of the effects of lipid mediators in inflammatory skin disease.

The articles are all of a high standard and from good laboratories. The apparent imbalance may reflect the interests of some of the editors and associate editors, who have a first class reputation in the platelet-activating-factor field. The editorial board, however, contains many eminent researchers from both European and North American laboratories and represents a much wider spectrum of interests — closer to that of the aims of the journal. It remains to be seen whether the journal will be able to broaden its base and attract

more contributions in the arachidonate and eicosanoid area or to be a focus for discussion on post-receptor signalling. It would seem that the journal's scope has been cast too wide and that it will have little hope, for example, of attracting many of the main lipoprotein papers. But if the same standards are maintained and a crisper definition of a lipid mediator is made, there is every prospect that the journal will make a useful contribution to our knowledge about these important compounds. □

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Healing arts

Michael T. Lotze

Biotherapy. An International Journal on Biological Agents. Editor in chief Huub Schellekens. *Kluwer*. 4/yr. Dfl. 332 (institutional), Dfl. 200 (personal).

Molecular Biotherapy. Editor Robert K. Oldham. *Butterworths*. 4/yr. North America \$145; elsewhere \$175.

ONE of the fables that exists in the medical collective consciousness is the story of the young house officer who regaled one of his colleagues with the notion that he wasn't very good at diagnostics in approaching human diseases, but felt that he had mastered the intricacies of therapy. The dilemma of many individuals facing a person with cancer has been just the opposite: not so much the diagnosis, but the intractability of the disease in the many people (more than half of those diagnosed) with resistance to conventional modes of therapy (usually surgery, radiation therapy and chemotherapy). The precise goal of the biotherapist is in large part to disturb the homeostatic balance between the host and the tumour. The availability of various recombinant cytokines, monoclonal antibodies and cell populations for adoptive therapy has excited the imagination of scientists, clinicians and the lay public alike.

Two new journals, *Biotherapy* and *Molecular Biotherapy*, international in scope and format, attempt to provide a forum for individuals working within this area. Because the borders of biotherapy are somewhat indistinct, evaluation of these biological agents in various other settings, particularly those of infectious disease and haematology, represent areas of investigation included in articles in the first four issues of both journals. At the outset, the reader should also know that I am on the masthead of an older (*Biologic Response Modifiers*) and a newer journal (*European Cytokine Network*) which have

scopes not dissimilar to the two I am reviewing here.

Attempting a rational programme of development from the dizzying array of biological agents available for laboratory, preclinical and clinical studies is a formidable task. In the early 1980s, the Biologic Response Modifiers Program of the National Institutes of Health was established in Frederick, Maryland, at Fort Dietrich in facilities reportedly previously used for evaluating germ warfare. Early studies were initiated and promoted under the leadership of Robert Oldham and Ronald Herbermann, and out of these arose the *Journal of Biologic Response Modifiers*. This journal was adopted by the Society of Biologic Therapy as its official publication and has entered a more mature phase, with monthly issues, in a larger format and with articles cited in the *Index Medicus*. Oldham, who initially steered this vehicle through its embryonic stages, has moved on to assume the helm of *Molecular Biotherapy*. This journal, as well as its counterpart, *Biotherapy: An International Journal in Biological Agents*, must be judged not only by their predecessors but also by their appearance and their promise for the future. Each of the journals is issued quarterly and has geographically placed editors, both in the Orient and the Occident. Both contain a series of articles of varying quality, typical of early start-ups that have not yet matured.

It isn't clear what format *Biotherapy* will eventually adopt. The earlier issues, starting in 1988, carried articles without subsection headings, ranging from the evaluation of recombinant cytokines in murine models of cancer and infectious disease to the role of Chinese herbal derivatives on superoxide scavenging, and the role of cholera toxin in inducing autoimmunity. Various reviews are included, discussing individual cytokines or areas as broad as biotechnology in Europe. The most recent issue I was sent for review includes articles focusing on the potential therapeutic uses of interleukin-1, edited by Charles Dinarello and Ruth Neta. This kind of assembled information provides a very useful review of the current status of this particular area and has significant advantages in reviewing this fast-moving field over textbooks, which have an obligatory one to three years of production delay. It is unclear whether such focused reviews, some of which are written by as many as 15 investigators, will become the exception or the rule in this journal.

Molecular Biotherapy is more differentiated in its organization: there are editorials and/or commentaries, largely by Oldham, a section entitled "review" and a series of papers. Patent reports, meeting reports and a calendar are variously included in different issues. "Commentary" provides a vehicle for Oldham to

discuss his assessment of biotherapy and the means to test it in the clinical arena. Last year, interleukin-2 was approved for clinical use in Europe. This summer, the FDA approved interferon- γ for the treatment of chronic granulomatous disease, and provided an initial stay of approval of interleukin-2 for the treatment of renal cell carcinoma. The Recombinant DNA Advisory Committee also approved the first studies attempting to perform gene therapy, in which the genes encoding two "biologics", adenosine deaminase and tumour necrosis factor, will be used.

There are ample reasons to think that this field will expand and require the kind of forum provided by these journals. Much of the current excitement that pervades modern biological sciences, however, has developed because of the reductionistic approach taken to isolating and

identifying the molecular species that appear to be the prime movers in biology. The introduction of molecules such as these, which have been so painstakingly dissected in recent years, back into the complex biological systems from which they arose, and to do so in a way which is formalistically and scientifically sound, represents both the problem and promise of the field and these new journals. Their success or failure in moving beyond their so-far inadequate contents will depend on their ability to apply the same rigorous standards as the more mature scientific disciplines (and journals). □

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Viable offspring

John Aitken

Molecular Reproduction and Development. Editor Ralph B. L. Gwatkin. Wiley-Liss. 6/yr. US \$100 (institutional), \$50 (personal); elsewhere \$116 (institutional), \$66 (personal).

Reproduction, Fertility and Development. Chairman of Editorial Board J. K. Findlay. CSIRO. 4/yr. Australia and New Zealand \$A120; elsewhere \$US120.

IT WOULD be difficult to imagine a more important area of biology than reproduction. A plethora of diverse global problems, including overpopulation, infertility, childbirth, HIV transmission, animal production and the preservation of endangered species, all fall under the umbrella of reproductive research and are dependent on advances in our fundamental understanding of this process.

Given its importance, it is perhaps surprising that reproductive biology, particularly the reproductive biology of mammals, has been such a neglected area of biomedical research. For much of the recent past, the subject has been dominated by endocrinologists who, while scoring a conspicuous success in providing the scientific basis for the contraceptive pill, have spent much of the intervening decades in an obsessive pursuit of hormonal profiles. The result has been a wealth of detailed technical data describing the vagaries of the hypophyseal gonadal axis in every sanguineous species from the aardvark to the zebra, but only a limited insight into the mechanisms regulating gonadal function.

A harsh generalization, perhaps, but it is undeniable that this whole-animal, hormone-orientated approach to reproductive research has, at last, reached its climacteric. Its place is gradually being

taken by a more fundamental, molecular approach, in which emphasis is placed on the cellular mechanisms regulating reproduction, rather than a description of circulating hormone levels *per se*. These two new journals are intriguing in that they reflect this division in reproductive science between the traditional, endocrine-based approach and the influence of the recent intrusion of molecular and cellular biology into this area.

Molecular Reproduction and Development firmly declares itself in the latter category. There is an unfortunate fashion among new journals to seek respectability by including the adjective 'molecular' in the title; titles such as *Molecular Andrology* and *Molecular Endocrinology* abound and it can only be a matter of time before the *Journal of Molecular Sexual Practice* appears. The question is, then, to what extent is *Molecular Reproduction and Development* the genuine article? That it can be deduced from the quality and international scope of its editorial board and the fact that Ralph Gwatkin, who previously created the excellent journal *Gamete Research* for Wiley-Liss, is its editor-in-chief. *Gamete Research* has been incorporated into the new journal, and as this area of reproductive research is, by its nature, dependent on a molecular, cellular approach, the fusion is both rational and effective. The papers I feel competent to judge seem to be of uniformly high quality and I have no doubt that this journal will fill an important niche in reproductive biology and, moreover, be important in shaping the future of this discipline.

I also hope that *Reproduction, Fertility and Development* will find a niche in the future landscape of reproductive biology. This Australian journal, with an all-Australian editorial board, is published by the Commonwealth Scientific and Industrial Research Organization of Australia and it contains, in the main, papers



A mouse embryo at 15 days of gestation (from *Molecular Biology of the Cell*, published by Garland).

from Australian laboratories. It has an all-encompassing brief in that it solicits original articles, reviews and comments on all clinical and scientific aspects of reproductive and developmental biology as well as reproductive endocrinology, including relevant aspects of puberty and lactation. The subjects covered are extremely diverse, although many, if not most, of the contributions exhibit a traditional, comparative endocrinology lineage, characteristic of the well-established journals in this field such as the *Journal of Reproduction and Fertility* and *Biology of Reproduction*. Not surprisingly, however, there is an increased emphasis on marsupial reproduction in the Australian example.

Many of the papers are excellent, reflecting the fact that Australia has, in recent years, led the world in several key areas of reproductive research, most famously in *in vitro* fertilization but also in areas such as the endocrinology of inhibin. But some of the papers on other topics in the first few issues are less than vintage, and would have struggled to see the light of day in any of the mainstream reproduction journals.

Herein lies the dilemma. *Reproduction, Fertility and Development* could develop into a useful organ of communication between Australian laboratories, reflecting the unique interests of the local scientific community. But there is a danger that it will become a repository for second-class papers which either have been, or would have been, rejected by the main journals in the field. A stringent editorial policy will be needed if this journal is to retain its quality, appeal — and viability. □

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Watch this space

Eric A. Barnard

Journal of Molecular Neuroscience. Co-editors Paul J. Marangos and Julia M. Polak. Birkhäuser. 4/yr. US \$131; elsewhere \$140 (surface mail), \$161 (air mail).

THE number of journals with an emphasis on molecular neurobiology is now noticeably increasing. As this is probably the most rapidly growing field in the whole of present day biology, there is justification for this recent proliferation: for example, the editors of *Neuron* (established 1988) already state that they can now take only a relatively small fraction of the papers in that field which they deem meritorious.

There will surely be a growing demand for more space for this subject-matter for some time to come, if it is in a journal of high repute. I do not join those who lament (for example in a couple of last year's new journal reviews, of the sour grapes variety) over the recent multiplication of journals in this field, when it is at such a stage of exponential growth: it has yet to be demonstrated that the molecular neurobiology periodical market is anywhere near being well enough catered for. In the present state of excess demand, idiosyncratic or coterie editorial preferences appear in some places, and more competition is the only effective answer.

Reputation is necessarily built slowly by a new journal, and the only quick alternative is for it to start out as the offshoot of a long-established and well-regarded periodical machinery of a well-established publisher in that field. The *Journal of Molecular Neuroscience* did not start off with this silver spoon and Birkhäuser is not a household name in biomedical science publishing. So it has a long road to travel, and has had to begin in quarterly form and with an average of only seven papers per issue. It will need to grow to a fuller extent and to a monthly appearance before it can make a real impact.

It has a good prospect of growing to offer such real competition. The editorial board is widely based in subspecialties and hemispheric distributions, and there is a good balance between eminent experience and youthful enterprise. The papers which the journal has attracted so far are for the most part interesting and of good quality. They include a few of the archival type to be expected for inaugural contributions, for example a paper by 13 authors which simply documents, very soundly but with not too fascinating a conclusion, that mitochondrial DNA has no significance in the inheritance of Huntington's disease.

The editors state in the second issue of volume 1 that they wish "to incorporate

brain protein biochemistry as a major part of this new journal" and they contrast this aim, rather scathingly, with "simply" publishing "sequence data and cloning results". Cloned sequences are, of course, now telling us a great deal of what is new in cellular neurobiology. Fortunately, the subject matter of the papers so far published shows that the large editorial advisory board is disregarding that dubious distinction. Its members certainly have the range and ability to make this, in due course, another journal of high standards and wide repute in molecular neurobiology. There is nothing so far to suggest that they will not achieve this, and neither potential contributors nor readers need fear that, if they succeed in bringing it up to the top class, there would then be too great a degree of competition in this field. This has the potential to be a journal for all neurobiologists to watch and to develop. □

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Good behaviour

A. Richard Green

Behavioural Pharmacology. Editor Paul Willner. *Clinical Neuroscience*. 6 issues/volume. *European Community* £87.50 (institutional), £45 (personal); elsewhere £100/\$185 (institutional), £55/\$100 (personal).

THE list of journals encompassed by the broad heading of psychopharmacology grows ever longer, and some titles are struggling somewhat to obtain enough good submissions and therefore flourish. It might be argued, therefore, that this is not a good time to launch yet another. But in its first year, *Behavioural Pharmacology* seems to have attracted a reasonable number of quality papers, and this has to bode well for the future.

The question arises as to reasons for this early success, given that the journal is neither tied to a learned society (unlike, for example, *Journal of Psychopharmacology* or *Neuropsychopharmacology*) nor issued by one of the large international publishing houses. A glance at the editorial board suggests one explanation. It is pleasingly international, and although the editors are senior, all have clearly been chosen because they are still in the thick of research — not just because they are names which might look impressive inside the front cover. A second explanation is perhaps contained in the line printed under the title on the front cover: "an international forum in which behaviour and pharmacology receive equal attention". Many pharmacology and psychopharmacology journals do not

seem to be appropriate for papers on physiological psychology, whereas journals on the latter subject often publish little on drug effects. In other words, a niche exists for this new publication.

Behavioural Pharmacology publishes original articles, reviews and commentaries, and the September 1989 issue contained abstracts from a workshop held under the auspices of the European Behavioural Pharmacology Society. The journal also accepts clinical papers, but few have appeared so far. Each issue is well produced, but the cover is old-fashioned and is not going to stand out on the library shelf and attract the casual browser. The journal does not print either submission or acceptance dates, so potential contributors cannot see how long it is likely to be before their work would appear in print.

In the first few issues there were a substantial number of research reports by members of the editorial board or members of their departments. Only time will tell whether other behavioural pharmacologists will submit their best work to this new journal, and it is only then that one will be able to say that this journal has a secure future as a major "international forum". Nevertheless, at present, the signs are promising. □

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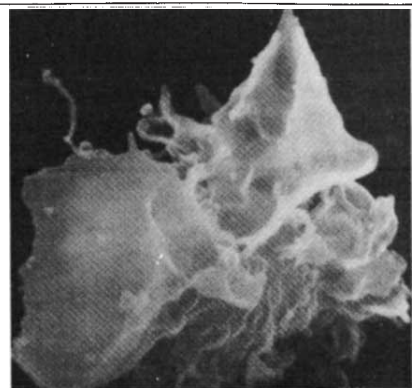
T cells or T shirts?

Peter Parham

Seminars in Immunology. Editor in chief Michael Julius. Saunders. 6/yr. *North America* \$110 (institutional), \$60 (personal), \$45 (student); elsewhere \$130 (institutional) \$80 (personal), \$65 (student).

SEMINARS in Immunology was a new one on me — perhaps because the stacks of Stanford's medical library toppled in last October's earthquake and order has yet to be restored. Its genre, however, is familiar. With this journal, one of a series "seminars in X", Saunders adds its contribution to the increasing selection of publications that distil the primary literature for those who create it. In immunology, these titles include *Immunology Today*, *Annual Review of Immunology*, *Advances in Immunology*, *Current Opinion in Immunology* and *Immunological Reviews*.

The flavour of *Seminars in Immunology* is most like *Immunological Reviews*, in that each issue is devoted to a single theme with relevant research being discussed by those who did it, or at least supervised its execution. Unlike *Immunological Reviews*,



Veiled threat — scanning EM of a mature dendrite cell showing two flattened lamellipodia or 'veils'.

which is single-handedly edited by Göran Möller, each issue of *Seminars in Immunology* involves a guest editor — an internationally acclaimed guru in the particular field. This I discovered makes sense as, according to the *Oxford English Dictionary*, a seminar is “a select group of advanced students associated for special study and original research under the guidance of a professor”. So far, the topics — T- and B-cell interactions, transgenic mice, T-cell development in the thymus and transmembrane signalling in lymphocytes — have covered active, rapidly moving areas of immunology, and the guest editors (respectively Charles Janeway, J.F.A.P. Miller, Jonathan Sprent and Gerry Klaus) have enrolled, with few exceptions, students of the highest calibre. Such “students” are not to be dominated by their professors and their essays are diverse both in style and scope, ranging from the overtly didactic to the demanding and impenetrable. In general they provide a snapshot of one laboratory's view of the universe it inhabits, and as such *Seminars in Immunology* is unlikely to benefit real students, new converts or the inquisitive heathen.

Most of these essays are full of meat, are frequently self-centred, and assume a familiarity with the notorious vernacular that makes immunology suspect to other biologists. Moreover, there are enough typographical immunoballs to perturb and potentially disrail the uninitiated: an amusing example being Allison and Raulat's successive designation of “the other” type of T cells as gd, g/d and $\gamma\delta$, evoking a not implausible evolution from the righteous through the indecisive to the pagan. Other features that will deter teachers from using *Seminars in Immunology* are the tentative typeface, the predominance of uninspired computer-made line drawings, poor reproductions of photographs and the quaintly heraldic cover with its fake electron micrograph of a tubercular antibody molecule. The last would probably look good on a T-shirt.

With diminishing money available for their research, many immunologists are

writing more and reading less. Numerous grants must be written as the chances of success for any given application decrease. These applications require bolstering with primary publications and, as these represent increasingly thinner slices of the salami, their significance and the insight to be obtained from reading them go down. Uncontrolled positive feedback between these two processes can set in, with the endpoint that once renaissance men and women become so enmeshed in writing their own grants and papers that they never read anything else. (This syndrome should be clearly distinguished from an older and highly esteemed condition, characterized by the voluntary preference to write rather than read the literature).

Saunders seems to appreciate this dilemma because *Seminars in Immunology* is tailor-made for those who find themselves snarled in its grasp. All the essays, irrespective of length, are heavily referenced — providing many useful citations, access to other reviews and entries into the primary literature, should one want to go that far. With each successive

issue of *Seminars in Immunology* there has been an approximately linear increase in the mean number of references per essay (57, 59, 68, 72) and, with an annual output of six issues, I project that about 350 references per essay will be attained by the turn of the century. In addition, the essays themselves provide excellent workhorse citations as each permits reference to any result, idea or slip of the tongue emanating from a major immunology laboratory. On the other hand, there is risk for prospective authors in writing such essays, in that they will encourage others to reduce their oeuvre to a single citation. Such apprehensions can undoubtedly be overcome by the persuasion of an appropriately armed guest editor. In this regard it is of interest that, contrary to what one expects from a new scientific journal, only one of the eight members of the editorial board has contributed to the first four issues of *Seminars in Immunology*. □

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Risk and regulation

Francesco De Matteis

Chemical Speciation and Bioavailability. Associate editors Douglas Craft (US), Margaret E. Farago (UK) and T. M. Florence (Australia). *Science and Technology Letters*. 4/yr. US \$140 (institutional), \$60 (personal); elsewhere £70 (institutional), £30 (personal).

THE environmental impact of chemical pollutants such as potentially toxic metals is best appreciated by measuring the specific chemical forms in which the metal is present in the environment rather than its total concentration as, needless to say, different compounds of the same element may vary considerably in chemical and biological activity. The basic concept that different compounds have different behaviour is of course fundamental to all aspects of chemistry, including those pertaining to biological disciplines, such as biochemistry, pharmacology and toxicology, and applies equally well to environmental sciences. In practice, however, analytical difficulties in measuring specific chemical forms have often meant that in environmental studies, biological risk assessment and related regulatory aspects the chief emphasis has had to be placed on the total concentration of a toxic metal, rather than on trying to define and measure its potentially active forms.

The main purpose of this new journal is therefore to remedy the situation by encouraging research into the way in which the chemical form of potentially toxic metals influences their behaviour,

including biological uptake and ecological fate. Contributions are encouraged from analytical chemists and environmental biologists alike, and also from scientists interested in engineering and regulatory aspects, with a view to providing a multi-disciplinary forum for exchange of information in this area of applied research. The ultimate aim is to improve the chemical understanding of environmental issues and to provide more valid and scientific criteria for risk assessment to human health.

Although one can only agree with these general aims, time will tell whether, and to what extent, the journal will be successful in achieving them. The issues made available for review suggest that the editorial board needs to attract more high-quality papers for the journal to establish itself on a secure footing.

I am doubtful about the word “speciation” in the title: this word is rarely encountered in the literature (I myself had never met it before, neither had any of my colleagues); its meaning is not defined and I am not clear in what way it differs from the more commonly used word “species”. As far as I can gather, it is meant to convey a variety of meanings — not only different forms of the same compound varying in oxidation state, charge, whether bound to an inert matrix, and so on. It is possible that “speciation” is a household word in environmental chemistry circles; if not, could it be defined in the first page of the journal? This suggestion has also been made by a member of the editorial board in a published letter to the editor. □

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Political science

T. J. Cole

American Journal of Human Biology. Editor in chief Francis E. Johnston. Wiley-Liss. 6/yr. US \$90; elsewhere \$114.

THE start of modern human biology is generally dated to the growth studies of Buffon and de Montbeillard in the latter half of the eighteenth century. The journal *Human Biology* appeared in 1929, and in 1974 the British Society for the Study of Human Biology started its own *Annals of Human Biology*. Both journals have been consistently successful, but why in these straitened days should a new journal appear in this small niche of the market?

The answer, sadly, is less to do with science than with politics. In 1986 there was a disagreement between Frank Johnston, the incoming editor of *Human Biology*, and the publishers, Wayne State University Press, and this led the Human Biology Council, which had until then recognized *Human Biology* as its official publication, to take its custom elsewhere. The result is the *American Journal of Human Biology*.

The first issue appeared in January 1989, and there are to be six issues a year. The journal is printed to a high quality on glossy paper, though the font is rather heavy for my taste. The format is conventional, with a dozen or so full-length articles plus book reviews. The Human Biology Council also publishes reports of its meetings. During 1989, four of the issues included symposia proceedings on growth and maturation; evolutionary, biosocial and cross-cultural perspectives on ageing; applications of molecular biology to issues in human biology; and influences of nutritional status on growth. These give a good idea of current research interests in the field.

Taking the fifth issue at random, the median time to publication was five months from acceptance and nine months from submission, perhaps slightly better than the competition. The quality of papers is generally very high, and it is clear from the authorship that several prolific research groups have switched their allegiances from *Human Biology*.

This raises the important question as to whether the market-place is big enough to support three journals in this field. *Human Biology* has tacitly answered this by announcing a switch in emphasis from general human biology to population biology and genetics. Judged by the first 18 months, the *American Journal of Human Biology* should continue to thrive, just as its predecessor has for 60 years. □

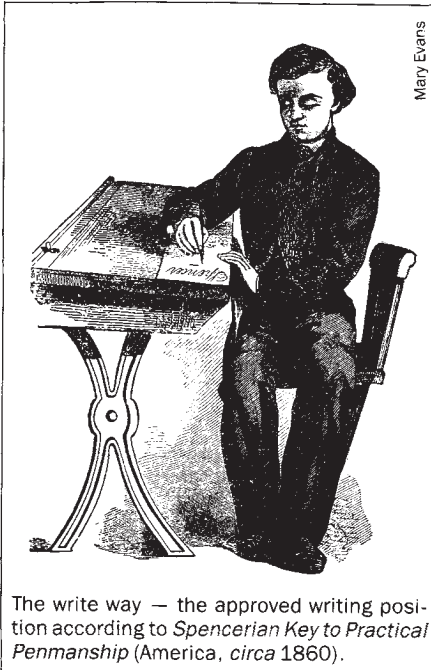
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Pen and ink

Paul Fletcher and Philip Smith

Reading and Writing. An Interdisciplinary Journal. Editor R. Malatesha Joshi. Kluwer. 4/yr. US \$102.50 (institutional), \$49 (personal); elsewhere Dfl. 210 (institutional), Dfl. 100 (personal).

READING and writing are areas of cognitive functioning of great importance to society. We would like to know more about how these skills develop, and what goes wrong in those individuals for whom they fail to develop adequately or who lose either of them in whole or in part. A new journal whose purpose is to publish



The write way — the approved writing position according to *Spencerian Key to Practical Penmanship* (America, circa 1860).

research pertaining to the “processes, acquisition and loss of reading and writing skills” therefore deserves scrutiny. However, in the context of a very extensive literature, particularly on reading and spelling, published in the past 20 years in general psychological and educational journals as well as in specialist sources, a newcomer will have to work hard to establish an individual voice. A possible contribution, half-promised by the title, would be to give writing a research priority equivalent to that of reading. Writing (apart from its spelling component) remains a relatively mysterious and neglected process.

On the evidence of the early issues, this has not yet happened. Of the 25 articles in the issues we received for review, more than half concern reading only. Of the rest, only two are wholly concerned with writing. One of these, on backwards writing, may be thought to be addressed to a somewhat *recherche* skill. The other, which examines the grammatical deficiencies of learning-disabled students’ writing,

is of considerable potential interest, but could have benefited from an analytical framework somewhat more informed by developments in modern linguistics. *Reading and Writing* is subtitled “an interdisciplinary journal”, and its editorial policy statement makes it clear that its purpose is to cut across the artificial boundaries that the different disciplines interested in literacy can erect. The early issues suggest that this is yet to be achieved.

The first volume certainly reflects recent work on reading: the remarkable recent expansion in reading research has arisen for various reasons, and the groups active in this area probably have very different aims in mind.

Cognitive psychologists have found written words ideal stimuli for studying perception, memory and attention. This is because the physical characteristics of words are readily specified, and many other properties of words, such as their frequency of occurrence, have been thoroughly recorded. Such research often uses reading as a means to an end, not as an end in itself, and the resulting research paradigms are not closely related to “normal” reading processes.

Cognitive neuropsychologists are interested in the functional architecture of the brain as revealed in brain-damaged patients, and have found written words convenient stimuli and responses, especially in patients whose communicative and cognitive abilities are limited. The most remarkable features of this group of subjects points to the modularity of reading processes: one patient (a deep dyslexic) might be able to guess the meaning of a word but not be able to read it out loud. Another patient (a surface dyslexic) might be able to read nonsense words aloud, but not real words (such as yacht) whose letter-sound correspondences are irregular. Yet another affected individual might be able to read only real words aloud, but not even the simplest non-word (this is referred to as phonological dyslexia). Observations like these have led to a view of reading as a skill requiring separate processes for converting writing to sound, accessing an internal lexicon, determining a word’s meaning and so on.

A further source of stimulation has been the realization that there is more to writing and reading than the 26 letters of the English alphabet, and work on language and scripts as diverse as Chinese, Hebrew, Japanese and Serbo-Croat have significantly extended our knowledge of word-recognition processes. Technological innovations have provided a stimulus also: machines to read books aloud to the blind, or to recognize handwriting, and ways of monitoring eye movements or modifying text in response to eye movements have generated substantial amounts of research

Traditional interest in reading, in particular answers to such deceptively simple questions as why some children fail to learn to read, has perhaps become less prominent in the field as a whole, though even here, work on 'phonemic awareness' in preliterate and illiterate has given an impetus to certain types of teaching methods.

Apart from the use of reading to study perception, attention or memory, all these strands of research are represented in this new journal, though in differing degrees. The modularity hypothesis, in relation to normal and impaired subjects, in both reading and spelling, is the best represented. There is little so far on languages other than English, or that takes advantage of computational advances (although the computer-based real-time investigation of reading by Jarvella and colleagues

is an excellent example of the genre). However, research in the 'traditional' questions of why children fail is surprisingly well-represented.

Although there is little sign yet, in the variety of topics, hypotheses and methodologies on show, of cross-disciplinary fertilization, it is fair to say that the first issues of this journal accurately reflect a diverse and thriving field. No doubt tighter integration will come, with a firm editorial hand. In the meantime, in these hard-edged times, there is surely something to be said for a journal catholic enough to find room to publish research on the effect of the study of Latin on spelling proficiency. □

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Casting neural nets

Stuart Sutherland

Neural Computation. Editor in chief Terrence Sejnowski. MIT Press. 4/yr. US \$110 (institutional), \$55 (personal); elsewhere \$124 (institutional), \$59 (personal).

JETAI : Journal of Experimental and Theoretical Artificial Intelligence. Editors Eric Dietrich and Chris Fields. Taylor & Francis. 4/yr. US \$120; elsewhere £65.

WITH commendable restraint, Terry Sejnowski, the editor of *Neural Computation*, has refrained from writing an editorial for the journal's first issue. One can, however, infer from the contents that he intends to cast his neural net wide. There are theoretical contributions on new and — so the authors hope — improved parallel distributed processing systems: these have little or no direct bearing on brain function, though many of the authors end with the rather unconvincing claim that they are putting forward "plausible biological models". There are papers that purport to solve particular problems like inferring the three-dimensional structure of a scene from the shading of its surfaces: they rarely provide complete solutions and it is hard to say whether the ones proposed bear any relation to those taken by the brain. Finally, there are papers directly modelling the activity of real neurons and even ones on neuroanatomy, describing, for example, aspects of cortical connectivity.

The journal publishes lengthy and for the most part intelligible reviews of particular topics. In an era of increasing specialization, these are welcome: those on neural networks for speech recognition and for learning are particularly useful. The accounts of original research are deliberately limited in length and are, for

no particular reason, called "letters", a nice compliment to *Nature*, which can — at least historically — justify the term.

Unfortunately, Sejnowski's misgivings about editorials are not shared by the editors of *JETAI* (a title stemming from the computing fraternity's belief that initials are more imposing than words). They view artificial intelligence as "a sober and circumspect scientific discipline — aware of its intellectual roots, presuppositions, and methods — that is at the same time, exciting, eclectic and justifiably optimistic". It is good to hear that things have changed, for in the past its protagonists have repeatedly been quite unjustifiably optimistic. Moreover, to be simultaneously sober and exciting is quite a *tour de force*. The journal covers all the topics included in *Neural Computing* as well as conventional AI and philosophical issues relating to the subject. It contains lengthy reviews as well as research papers, but many of the articles are vague prologomena to research rather than reports of work actually accomplished. Several papers, for example, "On designing a visual system", tell the reader at unnecessary length what he already knows.

One wonders where the subject is going and how long it will take to get there. From a technological standpoint, the most useful programs, for example expert systems, are those that do not attempt to simulate the workings of the human mind, for people are notoriously bad at thinking. On the other hand, no program begins to rival the skills at which people excel, such as recognizing faces or carrying out a conversation at a cocktail party in a room full of echoes.

For the most part, conventional AI has so far merely helped to delineate the nature of certain cognitive tasks, to uncover the environmental regularities that constrain the interpretation of patterns of stimuli, and to reveal previously unrecognized problems, such as that of detect-

ing the edges in a scene. Connectionism has different virtues. It has demonstrated that networks of neurons more or less randomly connected can show a hitherto unexpected capacity to learn quite complex tasks and can exhibit certain properties, like sloppiness, content-addressable memory and the ability to generalize, that characterize the human mind. But we are still a long way from knowing whether any of the many existing parallel distributed processing architectures corresponds to that used by the brain for a given task. Moreover, the proponents of parallel distributed processing ignore the fact that the brain is not simply a collection of neurons wired together in an excitatory or inhibitory fashion: it contains many neurotransmitters whose distribution is systematic and whose levels fluctuate.

Despite their use in clarifying our ideas, it is salutary to remember that so far both conventional AI and connectionism have only gone a small way towards the ultimate goal of understanding the human mind, but the humility one might expect this to induce is rarely displayed in either journal. Although the steps to understanding are small in both, those in *Neural Computing* are rather more solid. □

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Chipping in

D. J. Kinniment

Journal of VLSI Signal Processing. Editor in chief Earl E. Swartzlander, Jr. Kluwer. 4/yr. US \$173.50 (institutional), \$75 (personal); elsewhere Dfl. 305 (institutional), Dfl. 180 (personal).

International Journal of Computer Aided VLSI Design. Editor George W. Zobrist. Ablex, 355 Chestnut Street, Northwood, New Jersey 07648. 4/yr. US \$85 (institutional), \$32.50 (personal); additional charge of \$12 for overseas postage.

IT HAS been said that electronic design techniques are being affected more and more by the black hole of VLSI, by which ever more of a system is sucked onto the surface of a single chip, never to return. These two journals are concerned with various aspects of designing on silicon, including the VLSI chip designs necessary to process speech or images (*Journal of VLSI Signal Processing*) and the tools and techniques involved in producing efficient and correct chips (*International Journal of Computer Aided VLSI Design*). In their mission statements, both journals make the point that VLSI design is a practical engineering discipline as well as one which bases its advances on theoretical work,

and consequently many of the papers published here report on practical results and specific chip designs.

This is a useful approach, as it is often very difficult to compare widely different design techniques using different design tools for a range of applications, and publication of data on the results achieved is probably the best way of exposing the shortcomings of what seem to be promising theoretical ideas. A considerable proportion of the first year of publication of the *International Journal of Computer Aided VLSI Design* is dedicated to papers on the layout and interconnection of components on the chip surface. This is a problem to which well-known solutions exist, and where improvement, comparison and adaptation to new processing technology are the main areas of interest. Unfortunately this kind of article is read by only a few specialists, but a wider readership will welcome a special issue on VLSI computer-aided design in Japan which presents higher level work on a hardware description language, synthesis of circuits using knowledge-based techniques, and work on the effects of aberration in the optics used for defining the layer of masks used in chip manufacture.

The biggest problem now facing designers is the sheer size of the design task and the difficulty of getting from an overall specification, for example to reconstruct an image, to a detailed chip design of more than a million transistors, without making an error. There is, of course, no easy way of fixing errors once a chip is made, and there are no easy ways of checking its correctness fully before fabrication. Exciting developments in verification intended to address this problem are a hot topic, but are conspicuous by their absence in the first issues of this journal.

The material in the *Journal of VLSI Signal Processing* is of a high standard, with contributions from many of the well-known individuals and organizations in the field. Work on the fundamentals of arithmetic is represented as well as techniques for implementation of algorithms using systolic arrays, and actual chip designs. A new journal often has difficulties in maintaining a high quality of contributions while it is relatively unknown, and the approach taken here to maintain a good flow of publishable material is to devote the first two issues to extended and revised papers from the International Conference on Systolic Arrays held in San Diego in 1988. It will be interesting to see whether the editors can continue to attract the quantity and quality of papers necessary to sustain the journal in the future. □

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Hopeful recipe

John A. Campbell

Real-Time Systems. Editors in chief John A. Stankovic, Wolfgang Halang and Mario Tokoro. Kluwer. 4/yr. North America \$148 (institutional), \$65 (personal); elsewhere Dfl. 311 (institutional), Dfl. 160 (personal).

International Journal of High Speed Computing. Managing editors D. Gannon, A. Lichnewsky, Y. Muraoka and A. Sameh. World Scientific. 4/yr. US \$165; other rates on request from publisher.

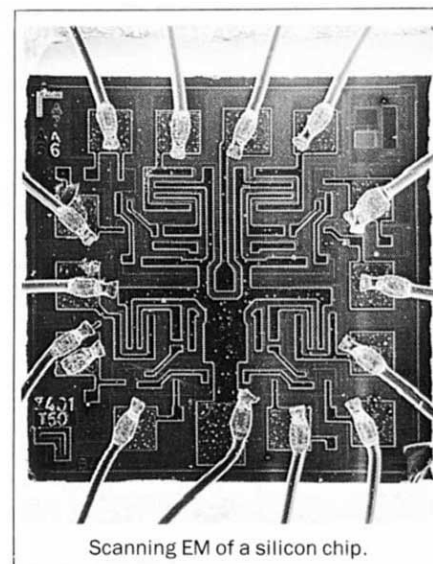
Impact of Computing in Science and Engineering. Managing editor Peter Deuffhard. Academic. 4/yr. North America \$80; elsewhere \$92.

Chance. New Directions for Statistics and Computing. Editors William F. Eddy and Stephen E. Fienberg. Springer-Verlag. 4/yr. North America \$50.95 (institutional), \$26.95 (personal); elsewhere DM 121.70.

THE financial arithmetic of producing a new and successful journal is much more attractive to a publisher than that of producing a successful research monograph. The only problem for a new journal is that in a time of increasing subscription costs and decreasing library budgets, how can enough paying customers be found to ensure success? To judge by the advertising material that I see, an important component of the ideal formula is to put computing into the recipe somewhere.

Publishers have now acted on this formula vigorously enough to give any good potential paper on computing a reasonable chance of seeing the light of day, in print, within a reasonable time. From being starved of journals only a short time ago, scientists with an interest in computing now tend to react to notices of each new one by asking (in the words of an anonymous mathematician who deserves better of posterity) which much-needed gap it is intended to fill.

One of the journals I review here clearly survives this test. *Real-Time Systems* does what its title suggests, which is to provide a welcome service in drawing together material that is otherwise not easy to find in one place. In addition, it emphasizes treatment of time-critical systems, and offers a home to several different subcultures which have not had much to do with each other in the past. For example, it contains papers by specialists in scheduling and time-dependent processes, who are now turning their attention to the distinctive problems of time-critical computing systems, and it recognizes that artificial intelligence also has a part to play in the subject, both as producer (of heuristics for scheduling, load-balancing and so on in applications too complex for



Scanning EM of a silicon chip.

exact methods) and consumer (of ideas that make it possible to estimate what resources and time are needed for a given computation to do its job — not a discipline that has been much in evidence in previous applied artificial intelligence).

The future of real-time systems will mix all these strands together, and move the subject into the foreground of computer science. The editors' choice of papers indicates that they appreciate that database technology and communications also belong in this picture. Probably the only relevant topic that is under-represented at present is distributed systems, but this seems to be a random effect, given the size of the sample of papers in the issues sent for review. *Real-Time Systems* has a good chance of being the main focus in print for the future development of its subject; both developers and users stand to benefit from reading it.

One way to ensure that a journal gets a good supply of high-quality papers is to make explicit efforts to round up collections of material on particular topical issues, either by raiding stocks of well-refereed papers from research workshops or by commissioning specialists to act as guest editors for individual issues. *Real-Time Systems* has exploited this heuristic already. Another journal to do it, with positive results, is the *International Journal of High Speed Computing*, which covers behaviour, design and experiences of use of parallel computing, whether for supercomputers or special-purpose systems based on components such as the transputer. From my own point of view, the number of interesting and useful papers in the first volume is distinctly greater than average. But the fact that an average can be defined is a possible problem. The journal's scope is so wide that it has many competitors in each part of its coverage: various *IEEE Transactions* series, the *Journal of Parallel and Distributed Computing*, the *International Journal of Parallel Programming*,

Molecular Simulation, and *Computer Physics Communications*, to name only those on my desk or in my department's reading room. Do we need another title? Probably not, though this opinion is consistent with the idea that the *International Journal of High Speed Computing* deserves to flourish at the expense of at least one of the journals just listed. I hope it maintains its first-volume standards and has the necessary luck.

The problem of competition will be even tougher in the case of *Impact of Computing in Science and Engineering*. The intention of this journal is to encourage communication among researchers and users of mathematical modelling, scientific computing and computer science, on the grounds that "the broad spectrum of techniques that are used nowadays to solve hard scientific and engineering problems is usually not represented within one journal". This is true, but the group of journals that covers the field (including titles mentioned above) tends to be displayed on adjacent shelves in good libraries: a reader interested in communication in print can find it just by moving his or her arms energetically while the feet remain fixed. A new journal in the area has to make its mark with papers that are, on average, more significant than those that its competitors carry. Unfortunately, there is no evidence from the first issues that *Impact* will do this.

Chance is the odd journal out in this

selection. The best short description is that it is a trade and tribal quarterly for US statisticians. It follows a model that Springer-Verlag has used to good effect for similar subjects already — news, historical features, articles in popular style but with a reasonable amount of technical content, interactions with public policy — though somebody who is not a statistical professional in the United States may be a little intimidated by the amount of American knowledge required to make sense of the material.

Nevertheless, if one is prepared to allow some concessions over baseball and US politics, it is a consistently good read. The tribal spirit that it demonstrates is instructive, and well worth the attention of the type of anthropologist who treats quasi-scientific tribes like psychiatrists as fit objects for study. (This is not a joke — I think.) The *and Computing* in the subtitle seems more exciting than it is in practice: the journal mainly covers evaluation of statistical software for microcomputers. The subtitle was probably chosen because of the "computing sells journals" formula. But one can forgive much in a journal that carries a regular gastronomic column, provided that its columnist takes a more enterprising approach to selecting restaurants on his next visit to France. □

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Hand in hand

Robert D. Gillard

Tetrahedron: Asymmetry. Editor in chief S. G. Davies. Pergamon. 12/yr. DM 550 (institutional), DM 153 (personal).

Chirality. Editor in chief Irving W. Wainer. Wiley-Liss. 4/yr. US \$120; elsewhere \$136.

WHEN the Abbé Galiani said, a hundred-odd years ago, that "*Les dés de la Nature sont pipés*", he spoke of the difference already well known between natural and synthetic substances (dice, with their 1,2,3-spots clockwise or counter-clockwise are prototype handed objects). The first systematic attempt to discuss origins of the dominant handedness of natural molecules was Japp's lecture ("stereochemistry and vitalism") to the chemistry section of the British Association for the Advancement of Science in 1898. He concluded that asymmetry begets asymmetry.

This existence and influence of handedness in such natural molecules as amino acids, sugars, terpenes and alkaloids (and other drugs) has been a subject of interest and use for many years. The remarkable growth in research on handed molecules during the past 15 years or so has built on

the efforts of the many scientists from Pasteur onwards, who had been fascinated by the diverse and diffuse phenomena of handedness.

These new journals (which will not lack for readers) recognize, in very different ways, this increasing activity in the synthesis, reactions and uses of handed molecules. Indeed, they are not alone: for example, the almost equally new-born *Journal of Molecular Recognition* (reviewed by Tom Blundell last year in *Nature* **341**, 357; 1989) also focuses on differences in such interactions as right-right versus right-left (or shaking hands as against holding hands). The UK Science and Engineering Research Council even has a special research initiative with the self-same title, molecular recognition.

Tetrahedron: Asymmetry is edited by S. G. Davies, who is at the Organic Chemistry Laboratory, Oxford University. Concerned chiefly with organic asymmetric synthesis and characterization of optically active molecules, it is (as one might expect from its *Tetrahedron* ancestry) directed towards organic — and perhaps organometallic — chemistry. This area is currently so active that it makes sense to provide a single focus for workers striving to increase optical purities of synthetic products. This objective has, of course, always been central to mainstream chemi-

stry (and will certainly continue to be), and many organic chemists may think it a pity to separate it from general synthesis.

Chirality has three editors based in institutions concerned with pharmacology and the like. Its clear intention differs from that of *Tetrahedron: Asymmetry*: it is to make pharmaceutical and cognate scientists more aware of the importance of handedness of molecules. The word chirality (compare chiropody, chiropractor and chiromancy), due to Kelvin, is merely a more general description of handedness.

One cause of the upsurge in work on the handed molecules is the grim reminder given by thalidomide that effects on living beings of enantiomers often differ sharply: one hand of this molecule had the desired effects, but the other was apparently teratogenic. The wrong hand was not merely "isomeric ballast". Similarly, one hand of penicillamine is beneficial in the treatment of Wilson's disease of copper metabolism, whereas the enantiomer has unpleasant effects, and benoxaprofen (a racemic anti-inflammatory drug) was withdrawn in 1982. The Kefauver-Harris amendments to legislation in the United States arose from the same concern as that which has motivated the foundation of this journal. *Chirality* covers a novel and valuable area, though one or two papers do seem irrelevant to pharmacy and so rather out of place. It is well produced on quality gloss paper, very useful for weighing out materials.

At the most recent meeting of the British Association, the chemistry section again held a session on chirality. Professor Stirling handed out caraway seeds and spearmint chewing gum. These flavours, quite distinct to our taste detection system, itself handed, apparently stem from the enantiomers of a single terpene. That kind of observation underpins the fascination and usefulness of this field of handed molecular interactions manifest in so many areas of science and art. Both these journals will serve a useful function through their different perspectives on handedness.

I must raise two quibbles. First, both journals use "asymmetry" in their titles when (*pace* Pasteur) they mean "dissymmetry". Both contain papers describing molecules with plenty of symmetry, albeit axial rather than reflectional. Second, someone (preferably boards of editors of new journals) really must decide and tell us whether we are to use SI units or not. No British high-school pupil has heard of a kilocalorie in a science lesson for 10 or more years, yet both these journals use these units unexplained. □

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From hydrogen to uranium

Robert W. Cahn

Chemistry of Materials. Editor Leonard V. Interrante. *American Chemical Society*. 6/yr. US \$49 members, \$299 non-members; elsewhere \$54–62 members, \$304–312 non-members.

NO, nuclear weapons don't come into it. . . the heading is meant only to denote the catholic coverage of the periodic table intended by this new journal. Chemists in the United States have recognized that materials are widely regarded as an important object of scientific attention, and there are numerous signs that they want some of the action. Next month, the American Chemical Society is organizing a conference with the title *Frontiers of Chemistry: Materials by Design*, and a recent issue of the Materials Research Society's bulletin carries an interesting article on how undergraduate chemists can be won for materials science. A recent book edited by Mary Good, published by the American Chemical Society, popularizes biotechnology and materials science. This new journal is clearly part of this offensive.

The journal began publication in

January 1989, and in the first issue of volume 2 the editor writes about his perception of the journal's role. It is to serve as a forum for fundamental research at the interface of materials science, chemistry and chemical engineering. Processing gets special mention, as it often and very properly does nowadays in the United States. The difficulty of getting enough papers on polymers is the only warning note; editors of many other broad materials journals can only echo "amen". A special function of the new journal is cited as being to give a home to materials-oriented papers too "chemistry-oriented" for most materials journals. The editor knows exactly what he is aiming at.

Some papers that seem of interest well beyond the confines of chemistry laboratories are on: the ultrasonic irradiation of Cu powder; use of tris(trimethylsilyl)arsine to prepare GaAs and InAs; short- and long-range order in β' -alumina containing Na and Eu; magnetic properties of nanometre-scale iron particles generated by iron atoms clustering in cold pentane; anodic electrosynthesis of CdTe thin films; surface characterization and depth-profiling study of electrodeposited Cr films; deep anisotropic etching of tapered channels in (110)-oriented Si; rare-earth ions adsorbed onto porous glass; luminescence as a characterizing tool; and preparation, purification and densification of ZnS powder from organometallics.

Another treats polymeric precursors to boron nitride.

Even leaving aside those papers which speak mainly or exclusively to chemists, there is enough in these first two years' issues to show that there is such a thing as materials chemistry, and that the journal worthily represents it. It occupies a niche distinct from the competition, such as *Journal of Solid-State Chemistry* (with more of a focus on crystal structure and defects) and *Advanced Materials* (with more emphasis on review articles). □

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Extraction from the East

William Davenport and Paul Calvert

Metals Materials and Processes. Editor C. V. Sundaram. *Meshap Science Publishers, Lakshmi Building, Sir P. M. Road, Fort, Bombay 400 001*. 4/yr. India, INR 1,500; elsewhere US \$125.

AS THE title implies, this quarterly journal covers a wide range of topics from metal-extraction processes through materials applications to materials modelling and theory. It contains a good mix of review articles and research papers, the latter mainly coming from Indian researchers. The journal is being launched at an important stage in India's technological development and many of the articles represent key aspects of this development. Papers on the behaviour of nuclear-reactor construction materials and fuels are, for example, given special prominence.

An important aspect of the journal is the emphasis it gives to materials processing. The core of materials science used to be seen as the relation between microstructure and properties. The new vision is that processing is a third main theme. What is not yet clear is how we can extract general principles from the details of specific methods for synthesis or shaping of materials. The editors acknowledge the importance of processing in obtaining enhanced materials properties and they are actively seeking papers in the processing area. Sol-gel processing and powder metallurgy metal-matrix composite processing are featured in the first year's issues. Papers on the thermochemistry of metal and metal compound production are also featured.

The first volume also includes papers on the extraction of primary aluminium and copper. These papers describe processing but seem to focus on improved methods of extraction rather than on methods

Merger matters

J. S. Rowlinson

Journal of Physics: Condensed Matter. Editor in chief R. A. Cowley. *Institute of Physics*. 51/yr. North America \$2,271; elsewhere £1,195. One section, **Liquids**, editor J.-P. Hansen, is available by separate subscription. 12/yr. North America \$271; elsewhere £142.

TWO years ago, the Institute of Physics decided that the division between the *Journal of Physics C: Solid State Physics* and *F: Metal Physics* was becoming difficult to defend. Many papers could have been assigned to either section and the institute thought also that it might be failing to attract other papers in the field of condensed matter because they did not fit comfortably into either class, as traditionally defined. These newer areas are surface properties, polymers, liquids, liquid crystals and glasses. The institute's solution has been to amalgamate sections C and F to form the *Journal of Physics: Condensed Matter*, and to identify a part of their new journal as *Liquids Papers*. Professor J.-P. Hansen of Lyon, supported by a separate editorial board, is in charge of the new subsection.

The institute also took the more

unusual step of extracting and publishing separately the papers on liquids, under the title *Liquids: a Section from Journal of Physics: Condensed Matter*. This subsection retains the original pagination. The parent journal appears weekly, with about 25 papers per issue, and the subsection is extracted monthly, with about 10 per issue.

The intellectual case for the amalgamation of sections C and F, and for the encouragement of the new borderline subjects, was clearly a strong one. The change has also been successful in practice; the papers published are numerous and of good quality. The liquids subsection has also got off to a good start, bolstered by some invited reviews on fashionable topics.

It remains to be seen how successful is the experiment of offering the liquids subsection both as part of the parent journal and as an independent journal. Almost all libraries will need the complete section on *Condensed Matter* if, indeed, they do not already subscribe to the whole of the *Journal of Physics*. But individual subscribers, tempted by the first year's offer of £59 for *Liquids*, will not have welcomed the price increase to £142 in 1990. □

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of obtaining enhanced materials properties. They might more usefully appear in an extractive metallurgy journal.

The journal is dedicated to alloys, amorphous materials, ceramics, composites metals and polymers. All are represented, metals and alloys the most strongly. Polymers have not yet received the attention they deserve.

The journal is obviously in the hands of an energetic editor and editorial advisory board. It is a vigorous addition to the materials literature. □

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In from the cold

Arthur K. Covington

Electroanalysis. Editor in chief J. Wang. VCH. 8/yr. US \$250 (institutional), \$95 (individual).

ELECTROCHEMICAL science can broadly be divided into mechanistic studies of electrodes in solution and applications of the better-behaved systems to elicit information (analysis) or provide useful technological products (batteries, fuel cells and, perhaps, cold fusion). Although it might be said that those with research interests in electroanalytical chemistry need a new journal like a hole in the pocket, *Electroanalysis* must be welcomed. The first issue, published in January last year, contained some particularly interesting contributions and the standard seems to have been maintained in subsequent issues.

This year the number of issues has increased from six to eight. Special issues will also appear: the first (April 1990) was on the important new topic of microelectrodes and contained ten feature articles and three short communications; it is available separately at £24 (hardbound). These contributions appear to have been specially commissioned and are not part of a conference proceedings.

The format is large (nearly A4), double-column, and clearly printed, not camera-ready typescript. Publication from submission is reasonably fast at 6–8 months.

Electroanalytical methods of analysis and electrochemical sensors are clearly areas of increasing interest. Whether Wang and his distinguished international editorial board succeed in creaming away from established journals such as *Journal of Electroanalytical Chemistry* (if it recovers from publishing on 'cold fusion'), *Electrochimica Acta*, *Analytical Chemistry*, *Analytica Chimica Acta*, *Selective Electrode Reviews* and the new divided *Sensors and Actuators Part B*, sufficient of

the important material for *Electroanalysis* to maintain and even improve on its initial standard remains to be seen. I shall certainly have to include *Electroanalysis* in my personal scanning lists from its present performance. For individuals it is not expensive, at least not at the moment. □

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Too much heat?

Peter T. Landsberg

Continuum Mechanics and Thermodynamics. Editors in chief K. Hutter and I. Müller. Springer-Verlag. 4/yr. DM. 298.

THE aim of this journal is to bring together interesting applications of continuum mechanics and thermodynamics, bearing in mind the advances in recent years in non-newtonian fluids, liquid crystals, gas mixtures, shape memory alloys, and so on. In this task it appeals to engineers, physicists and mathematicians to send their contributions. There is also a distinguished international editorial board. The second volume has just started with papers received in September 1988 and April, May and July 1989, on topics such as heat and mass transport and waves in viscous fluids. On the available evidence, this will be a good quality journal.

I cannot, however, disguise my opposition to new journals unless they are absolutely essential. And this journal is not essential. The whole subject area is adequately covered by *Archive for Rational Mechanics and Analysis* (Springer), *Acta Mechanica* (Springer), *Liquid Crystals* (Taylor and Francis), the *Journal of Non-equilibrium Thermodynamics* (de Gruyter) and others, and also by general journals like the *Journal of Physics*, *Physical Review* and the *Proceedings of the Royal Society*. My university library, which has a considerable reputation, has already stopped taking journals essential for research, including old and well-established titles. There is no chance of it subscribing to this new journal.

Adding journals of this type, despite the distinction of their editorial boards, is a major disservice to the academic community which they claim to serve. One has to remind oneself of the question repeatedly raised in recent years — is the existence of many journals "for the benefit of the contributors only, not that of the readers"? (See John Merriman's article in last year's journals supplement in *Nature* 341, 349; 1989.) □

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Making waves

Ian S. Robinson

Soviet Journal of Physical Oceanography. (A translation of the Russian language journal *Morskoi Gidrofizicheski Zhurnal*.) Editor in chief V. N. Ereemeev. VSP. 6/yr. DM 670, US \$395.

IT is all too easy for English-speaking scientists to measure the progress of their subject in terms only of what is published in English. As the publication rate of scientific papers seems to accelerate relentlessly, it is not surprising that we often lack the stamina to keep abreast of recent work published in other languages, expecting the important results to be offered to English journals anyway. By adopting such an attitude we risk losing sight of the evolving trends of subjects which have a strong indigenous base in countries with their own scientific publishing culture. Such a case is the study of oceanography in the Soviet Union.

Physical oceanography has a strong history among Russian-speaking scientists, but despite attempts at East-West collaborative experiments, I fear that many of my colleagues share my ignorance of what is happening within Soviet marine science. At a time when physical oceanographers throughout the world are being called on to play a key role in improving our understanding of global climate change, and when political barriers are suddenly falling away, there is more reason than ever to foster communication between Western and Soviet ocean scientists.

These thoughts sprang to mind as I read through the first four issues of the *Soviet Journal of Physical Oceanography*, and discovered that Soviet oceanographers are concerned with very similar problems to their Western counterparts, but often offer a different perspective on their solution. For example, the authors of several papers examine the dynamics of meso-scale eddies and turbulence in the upper ocean, but the analytical and numerical approaches are rooted in the literature of Soviet applied mathematics. Experimental results are presented from observations in parts of the world's ocean which Western vessels rarely penetrate. Remotely sensed data come from more than the familiar NASA satellites. Thus, although I found nothing of outstanding scientific importance which would have been missed had this cover-to-cover translation of a Russian journal not been launched, I did find that it was the subtle differences in the way ideas are presented which stimulated new ways of thinking about our common oceanographic problems.

The extensive citation of other Soviet work also hints that there is much more to

be discovered in fields such as ocean optics and acoustics. It is a pity that the reference lists do not consistently denote whether the cited papers are in English or Russian. Another shortcoming was the absence of a date of either submission or first publication in Russian, so that the reader cannot judge how recent the results are. The most recent citations are a few years old, and the release of the first four issues of volume 1 has itself spanned nearly four years.

The subject matter does indeed cover all aspects of physical oceanography, theoretical and experimental, small and large scale, computational and analytical, remote-sensing and conventional ship

methodology. The papers are generally brief, written in a terse style which selects the key ideas and conclusions rather than presenting methods in detail, although not as abbreviated as some other translations of Russian marine journals. Some papers are written in lucid English, although the quality of translation is patchy. Thus the journal would not be very accessible to those new in the field of study, but for physical oceanographers this is a welcome newcomer which will surely repay regular inspection. □

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Insiders' viewpoint

Andrew S. Mackenzie

Oilfield Review. Executive Editor Henry N. Edmundson. *Schlumberger/Elsevier.* 4/yr. US \$128.50, Dfl.257.

OIL companies have relinquished control of many technologies important for the location and exploitation of oilfields to subcontractors. The subcontractors are responsible for innovation — and the oil

multinational service company — is the publisher of *Oilfield Review*, with Elsevier organizing printing and distribution. *Oilfield Review* is a demonstration of Schlumberger's involvement in the progress of many petroleum sciences.

The articles already published cover a stimulating range, from exploratory geophysics to corrosion control. They are all well illustrated, and readable by petroleum scientists from different specialist disciplines to those of the authors of the articles. Punchy summaries allow quick selection of material of interest. The level of coverage is designed for managers of petroleum science required to keep in touch

insights, such as the evaluation of prospects offshore from Norway. Others address phenomena important to all petroleum sciences from seismics to reservoir engineering, such as the anisotropy of sedimentary rocks.

The journal does smack slightly of a Schlumberger advert — almost all contributors are company employees. This one company surely cannot claim to monopolize all the good ideas. It needs to invite contributions other than the guest editorial from outsiders — the subcontractor must learn to subcontract. □

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Solid feature

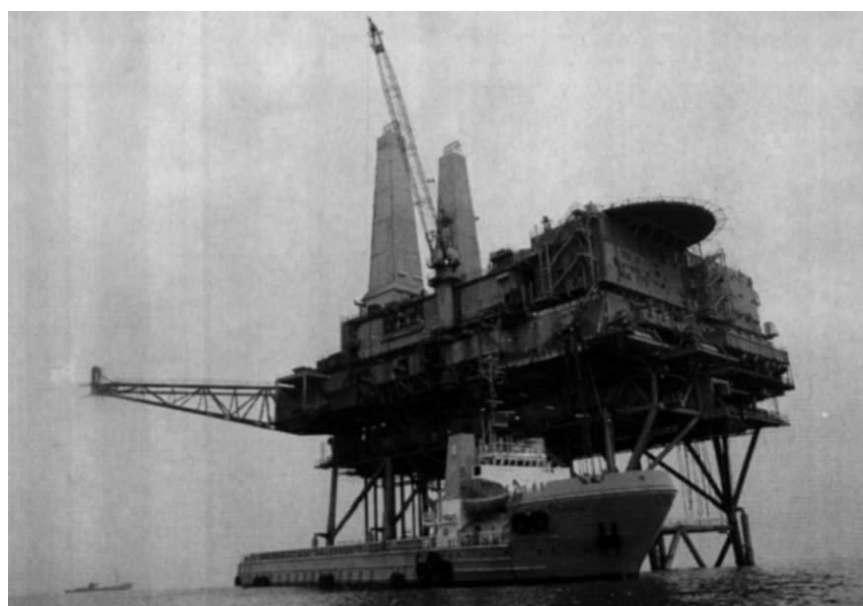
Alec Smith

Terra Nova. Editor in chief R. Muir Wood. *Blackwell Scientific.* 6/yr. North America \$224.50; UK £120; elsewhere £135.

Terra Nova is a geosciences journal with a difference, if only for the breadth of coverage in every issue. Spawned by the European Union of Geosciences, the only pan-European society for all those interested in the sciences of the solid Earth, *Terra Nova* goes to all union members as part of its annual subscription of £30. With more than 100 pages per issue, they get a bargain. (Non-member and institutional subscribers have to pay considerably more.) Though the union started in 1981, *Terra Nova* made its first appearance in 1989 and is issued bi-monthly.

Terra Nova is much concerned with European geosciences. It has its eye firmly on the integrated European Community of 1992 and beyond into the new post-cold war Europe from the Atlantic to the Urals, and a fair amount of the Middle East as well. Published entirely in English, it offers speedy publication of refereed research papers in all aspects of the geosciences (the list would be too long to reproduce here) as well as regular sections of news, reviews, correspondence, books, computing, expeditions, eyewitness accounts, events and a calendar of meetings. The pattern is repeated in each issue and subscribers can always look forward to features with anticipation.

The journal is not for those interested in only their own chosen field or part of a field of geoscience: the approach is broad yet challenging. It is not a journal for the layman, but it is worthy reading for non-geoscientists who wish to see what the subject is currently about. For geoscientists, it offers a stimulating insight into progress in the entire spectrum of the subject together with a fair discussion of the field's pan-European



A fixed oil production platform in the North Sea, owned by Union Oil, with supply ship alongside and safety boat in the distance.

companies are simply informed consumers. The economies of scale available to subcontractors who provide these services allow them to outperform the in-house facilities of large oil companies. The more successful subcontractors have swallowed others to create large multinational service companies which assist oil companies from discovery to production.

Schlumberger — the highly renowned

with new advances involving all disciplines of petroleum science and engineering — or for specialists wanting to broaden their horizons.

This journal is not just written by and for different kinds of specialists who never talk to each other. There are several articles about work that involves different specialists working together productively on a problem which needs different

political and social responsibilities.

One cannot fail to be impressed by the quality of the publication; not only by the depth of treatment but by the many topics already covered in the journal's brief existence. Two thematic issues have appeared in the eight issues to date: "Milankovitch cycles" (14 articles) and "Environmental geology and the marine dimension" (6 articles). Other splendid articles delighted me: the "Geology of the Bible" with many fine coloured plates and the well-merited reprint of Peter Gould's thought-provoking article "Tracing Chernobyl's Fallout" are but two of them.

This is a well-illustrated journal with many line figures and photographs. A particular feature (a house style?) is the use of reproductions of mediaeval woodcuts and nineteenth-century pictures, both as section markers and to illustrate the text where appropriate. This novelty may well wear off with time, however.

Much of the dynamism of *Terra Nova* owes a great deal to its editor in chief, Robert Muir Wood: his editorials are direct and thought-provoking (see, for

example, his piece on the refereeing system and peer review) and the balance of each issue has been splendid. The journal sets out to achieve, and seemingly has succeeded, in making geoscientists communicate on more than what all too often are their narrow and personal interests while ensuring a rapid publication of research papers. The geographical spread of authors and frequent multinational co-authorship of papers to date certainly reflect the pan-European and broader appeal while the wealth of subjects reveal the breadth of geosciences today.

Since more than 2,000 participants attend each biennial European Union of Geosciences meeting in Strasbourg, *Terra Nova* should be assured of a sound subscription base. It deserves to succeed, for it will do much good for European research as well as for all those who care to read its papers. □

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Icing on the cake

John Splettstoesser

Antarctic Science. Editors in chief David W. H. Walton (life sciences and oceanography), Michael J. Rycroft (atmospheric sciences) and Michael R. A. Thomson (Earth sciences and glaciology). *Blackwell Scientific.* 4/yr. North America \$132.50 (institutional), \$75 (personal); UK £72.50 (institutional), £35 (personal); elsewhere £72.50 (institutional), £42 (personal).

THE onset of large-scale research programmes in Antarctica during the International Geophysical Year of 1957–58, followed by the creation of the Antarctic Treaty in 1959 (ratified in 1961), and the subsequent continuation of research by many nations, produced a flood of published literature about this unique continent. Much of this literature was dispersed in traditional scientific journals and other serials, and proliferated with the addition of other countries to the original 12 treaty signatories. Control of this literature began in the United States in the early 1960s with the compilation of *Antarctic Bibliography*, a continuing series of volumes that includes annotated citations of all publications about Antarctica. Now we have an all-encompassing journal, *Antarctic Science*, devoted to the publication of research results in three broad disciplines — life sciences and oceanography, Earth sciences and glaciology, and atmospheric sciences. The editors of the journal, the first issue of which was

published in March 1989, are three distinguished researchers at the British Antarctic Survey in Cambridge, and are provided with guidance for content by an editorial advisory board of 19 people from 12 countries.

The geographical coverage includes

meeting reports, a noticeboard, book reviews and special issues. Articles on logistic operations, politics or history are not accepted. Volume 1 contains 37 articles (including reviews) and 3 short notes; the last issue of volume 1 includes a helpful index.

The British Antarctic Survey has taken the lead in starting a much-needed journal of Antarctic science that provides refereed, quality articles by investigators from many countries. This is a considerable challenge, inasmuch as the readership might conceivably consist only of authors' colleagues who are also involved in Antarctic research. Furthermore, there is always the prospect of losing subscribers because of the interdisciplinary content, which might include subjects outside the interests of some specialists. However, Antarctica has passed from the early research of the International Geophysical Year of a general or reconnaissance nature into matters of global consequence, involving depletion of stratospheric ozone, ice-core studies to detect changes in atmospheric chemistry, oceanic and atmospheric warming and possible melting of the Antarctic ice sheet, and investigations into the major carbon sink of the Southern Ocean. This is the strength of the journal, which I believe makes it necessary reading for a wide audience.

Finally, the quality of the scientific content of *Antarctic Science* is equalled by the



A drift blowing across sea ice at the British Antarctic Survey base of Rothera, Adelaide Island. The small hut is a pump house for a desalination plant.

Antarctica, the SubAntarctic (excluding the Falkland Islands, Tristan da Cunha and the New Zealand Shelf Islands), and the Southern Ocean. This generally conforms with the boundaries of the Antarctic Treaty coverage. In addition to review and scientific papers, *Antarctic Science* publishes guest editorials, short notes,

quality of its production, as confirmed by the fact that the journal received an award in a competition with 140 entries for typographical excellence in journal and serial publishing. □

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Flat Earthers

Peter J. Smith

Global and Planetary Change. A Daughter Journal of Palaeogeography, Palaeoclimatology, Palaeoecology. Editors Eric Barron, Sierd Cloetingh, Ann Henderson-Sellers, Diana Liverman, Michael Meybeck, Berrien Moore and Paolo Pirazzoli. Elsevier. 4/yr. North America \$135; elsewhere Dfl. 277.

THERE has been much talk of late among Earth scientists of global change, global systems and global interactions. NASA calls it Earth System Science, the aim of which is "to obtain a scientific understanding of the entire Earth System on a global scale by describing how its component parts and their interactions have evolved, how they function, and how they may be expected to continue to evolve on all time scales". The International Council of Scientific Unions (ICSU) calls its version the International Geosphere-Biosphere Programme (IGBP), which has almost identical aims. But these are merely the highest strata of the global infrastructure, capping a vast new sub-culture of committees, programmes, departments and publications.

The US Committee on Earth Sciences is defining a Global Change Research Program; the National Academy of Sciences has set up a Committee on Global Change; the National Oceanographic and Atmospheric Administration has its Panel on Climate and Global Change; and the IGBP has spawned a Scientific Steering Committee and innumerable "coordinating panels" and "working groups". Meanwhile, Rice University in Texas now has an Earth Systems Institute, while Pennsylvania State University has formed an Earth System Science Center. In the NCAR Advanced Study Program, the National Center for Atmospheric Research and seven other institutions are producing teaching modules on global change. ICSU is publishing a regular *Global Change Newsletter*, and the Office for Interdisciplinary Earth Studies in Colorado issues the somewhat more glossy *Earthquest*. And, of course, where scientists boldly go, journal publishers are never far behind. The American Geophysical Union produces *Global Biogeochemical Cycles*, and now Elsevier is publishing *Global and Planetary Change* (GAPC).

What can it all mean? Not a lot thus far, and certainly much less than the hype suggests. For one thing, there is little new here in principle. Earth scientists have always been interested in long-term global change — witness studies on worldwide changes in sea level, the growth of continents, compositional variations in the

atmosphere, the rate of change of radioactive heat flow with time, ocean-continent interactions, global tectonics, and much else besides.

Is it, then, simply some semantic reformulation designed to attract more financial support in hard times? Partly, perhaps even largely, but not entirely. There is little doubt that scientists' recent ability to link genuine Earth science research with dubious human influences and prophecies of doom (global cooling was the flavour of the 1970s, global warming that of the 1980s) has done wonders in prising open government coffers. On the other hand, through increased knowledge and advancing techniques there should, in theory at least, come a time when it should be possible to make some interesting, and perhaps even important, cross-disciplinary global connections.

That this point has barely been reached, if at all, is well illustrated by the first five issues of GAPC, the general aim of which is "to achieve an interdisciplinary view of the causes, processes and limits of variability in planetary change". The range of topics among the 30 or so pages is huge; to give some idea of the diversity it is perhaps sufficient to mention just solar evolution, solar and galactic influences on the Earth, the growth of the Earth's crust, long-term stability of the climate, the hydrological cycle, North American cloud variations, global sea-level changes, the quality of

rivers, changes in global carbon, soil contamination in urban areas, mass extinctions, plant evolution and palaeo-environmental modelling.

Yet interesting and of high quality as many of these papers are, the whole exercise rather misses the mark. For although the journal is conspicuously interdisciplinary, hardly any of the individual papers are multidisciplinary in anything but a trivial sense. Earth scientists write on Earth sciences, chemists on chemistry, biologists on biology, and so on; few, if any, connections are made. The international team of editors (which, incidentally, includes no Briton) might well argue that exposing work in one discipline to scientists in others is worthwhile in itself on the principle that it will increase the chances of cross-fertilization with benefit in the longer term, but I remain sceptical. Most people just don't have the time.

On the evidence of GAPC, Earth system science has not proceeded beyond the "component parts", and to suggest otherwise is merely a marketing ploy. As an interdisciplinary review medium, the journal itself is not without its usefulness, but as an advert for the brave new world of global interacting systems it falls rather flat. □

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Also submitted for review

THE following is a list of the journals that were eligible for review but that for one reason or another are not covered in the preceding pages. The list does not include journals sent to us that had not published enough titles to be considered.

Annals of Clinical Psychiatry. Official Journal of the American Academy of Clinical Psychiatrists (Elsevier).

Applied and Theoretical Electrophoresis. The Official Journal of the International Electrophoresis Society (Macmillan).

Applied Mathematics Letters (Pergamon).

Astronomy and Astrophysics Review (Springer International).

Discovery and Innovation (Sponsored by the African Academy of Sciences and the Third World Academy of Sciences; published by Academy Science Publishers, PO Box 14798, Nairobi).

Ecological Economics. The Journal of the International Society for Ecological Economics (Elsevier).

Ecological Psychology. A publication of the International Society for Ecological Psychology. (Erlbaum).

European Journal of Mineralogy (*Deutsche Mineralogische Gesellschaft, Società Italiana di Mineralogia e Petrologia and Société Française de Minéralogie et de Cristallographie*, in cooperation with

the European Mineralogical Union). *Frontiers of Medical and Biological Engineering* (VSP).

Games and Economic Behaviour (Academic).

International Environmental Affairs. A Journal for Research and Policy (University Press of New England).

Journal of Applied Psychology (Kluwer).

Journal of Cryptology. (Springer).

Journal of JASTRO. The Official Journal of the Japanese Society for Therapeutic Radiology and Oncology (Pergamon).

Journal of Molecular Endocrinology (Society for Endocrinology).

Journal of Population Economics (Springer International).

Journal of Substance Abuse (Ablex).

Laboratory Robotics and Automation (VCH).

MCSS. Mathematics of Control, Signals, and Systems (Springer International).

Military Psychology. The Official Journal of the Division of Military Psychology American Psychological Association (Erlbaum).

Schizophrenia Research (Elsevier).

Seminars in the Neurosciences (Saunders).

Trends in Endocrinology and Metabolism (Elsevier).

■ *Nature's* next review supplement will be Autumn Books, which will appear in the 22 November issue.

Please follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers for whom English is a second language and those who work in other fields. Please write clearly and simply, avoiding unnecessary technical terminology. *Nature's* staff will edit manuscripts to those ends if necessary. Contributors should check their proofs carefully.

Because of the competition for space, many of the papers submitted for publication cannot be accepted. For this reason, and because brevity is a great assistance to readers, papers should be as brief as is consistent with intelligibility. Please note that one printed page of *Nature*, without diagrams or other interruptions of the text, has fewer than 1,300 words.

CATEGORIES OF PAPER

Review Articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance but must not exceed six pages of *Nature*.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles should be accompanied by a heading of 50–80 words written to advertise their contents in general terms, to which editors will pay particular attention. A heading (printed in italic type) is not an abstract and should not usually contain numbers or measurements. The study should be introduced in more detail in the first two or three paragraphs, which should also briefly summarize its results and implications.

Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

Letters to Nature are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should not have more than 1,000 words of text or more than four display items and should not occupy more than two pages of *Nature*. The first paragraph should describe, in not more than 150 words, the origins and chief conclusions of the study. Letters should not have subheadings or more than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned, most are unsolicited. They are normally between one and four pages of *Nature* in length.

News and Views articles are intended to inform non-specialist readers about a recently published advance. Suggestions should be made to the News and Views Editor. Illustrations are welcome. Proposals for meeting reports should be agreed in advance.

Scientific Correspondence is for the discussion of scientific matters, including contributions published in *Nature*. Priority is given to contributions of less than 500 words and five references. Figures are welcome.

Submission. All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value \$5' to prevent the imposition of import duty and value-added tax (VAT).

GENERAL

All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

Colour Artwork. A charge of £500 a page is made for 4-colour figures. Inability to meet these costs will not prevent the publication of essential colour figures if the circumstances are explained.

Figure legends must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963–65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.